The Patent Office

PCT/GB99/03286

INVESTOR IN PEOPLE

09/806840

GB99/3286

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Andrew Gersey

Dated 8 October 1999

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

THIS PAGE BLANK (USP

Patents Form 1/77

Patents Act 1977
(Rule 16)

THE PATENT OFFICE
A
-5 OCT 1998
RECEIVED BY FAX

The Patent Office

060CT98 E395010-1 D02813
P01/7700 0.00 - 9821655.9

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

05 OCT 1998

The Patent Office
Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference — P57238M

2. Patent application number *(The Patent Office will fill in this part)* — **9821655.9**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Glaxo Group Limited
Glaxo Wellcome House
Berkeley Avenue
Greenford
Middlesex, UB6 ONN, United Kingdom

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — GB 473587003

4. Title of the invention — CHEMICAL CONSTRUCTS

5. Name of your agent *(if you have one)*

Fry Heath & Spence

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

The Old College
53 High Street
Horley
Surrey RH6 7BN

Patents ADP number *(if you know it)* — 05880273001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	43
Claim(s)	5
Abstract	–
Drawing(s)	8

THE PATENT OFFICE
A
-5 OCT 1998
RECEIVED BY FAX

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Fry Heath & Spence Date 05.10.98

12. Name and daytime telephone number of person to contact in the United Kingdom

Dr M.R. Hutchins - Tel: 01293 776880

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*

e) *Once you have filled in the form you must remember to sign and date it.*

f) *For details of the fee and ways to pay please contact the Patent Office.*

CHEMICAL CONSTRUCTS

This invention relates to chemical constructs for use in solid phase synthesis, and to methods of detecting and/or characterising the products of solid phase synthesis using the constructs.

Solid phase synthesis has been known for many years in the field of peptide synthesis and more recently has also been used increasingly for the synthesis of non-peptides.

Solid phase synthesis has found particular application in the field of combinatorial chemistry and the preparation of chemical libraries as potential sources of new leads for drug discovery, see for example Anthony W. Czarnik, *Analytical Chemistry News and Features*, June 1, 1998, pp 378A-386A, and *The Combinatorial Index*, Barry A. Bunin, Academic Press, San Diego 1998. A feature of combinatorial chemistry methods is that they enable very large numbers of different compounds to be prepared from a relatively limited number of molecular building blocks in a relatively small number of reactions. Combinatorial chemistry makes use of the "split and pool" approach in which a suspension of chemical starting material tethered to a solid support is split into N portions, each of which is reacted with a different reagent. The products of the N reactions are then pooled and mixed thoroughly, the resulting pool is split into N' portions and again each portion is reacted with a different reagent. This procedure can be repeated as many times as there are steps in the reaction sequence. Thus, for a three step reaction sequence, if the reaction mixture is divided into ten portions at each stage, each of the pools being reacted with a different reagent before recombining with the other portions, the total number of

compounds formed by the process will be $10^3 = 1000$. Thus it can be seen that by using the split and pool technique, a large number of different molecules can be synthesised using a minimal number of reactions (thirty in the case mentioned above).

Each of the solid supports (e.g. a resin bead) will have a single product molecule tethered to it and hence in principle each of the reaction products can be separated and analysed or subjected to biological testing simply by isolating each single solid support and cleaving the product from the support. However, the large numbers of compounds generated by combinatorial methods means that it can be impracticable to identify and characterise each compound. Consequently, the compounds usually are first tested, either on the solid support or after cleaving from the support, and only those compounds which show some biological activity are subsequently identified. In order to minimise the number of biological tests carried out, compounds can be tested in pools containing a predetermined number of compounds, the inactive pools being discarded and the active pools being subject to further investigation. The biological activities of the compounds can be analysed using high throughput automated assay techniques permitting large numbers of compounds to be analysed in a short time.

One of the problems facing the chemist is how to identify and characterise the various compounds in a combinatorial library since each compound will be present in the library only at very low concentrations, and there will usually be insufficient compound present on a given solid support to allow for both biological testing and identification of the compound. This problem has been addressed by providing each solid support with a coding tag from which the identity of the compound can be determined. Thus, for example, a coding tag can be built up in sequential steps on the solid support in parallel with the construction of the desired target compound, the coding tag reflecting the synthesis history of the product compound and being unique for each product compound. The coding tag is usually built up on the support using

chemical reactions of a type which are orthogonal to the chemistry used to build up the product compound, thereby ensuring that the coding units and product compounds do not become confused. Once the product compound has been tested, and its biological activity confirmed, the coding tag can then be decoded to allow identification of the compound.

In any solid phase synthesis procedure, whether or not part of a combinatorial procedure, it is important to be able to determine the optimal conditions for a given reaction step in a series of steps. It is also important to be able to monitor the progress of a reaction so that it can be determined whether a particular reaction has gone to completion. This is particularly important in a multistage solid phase synthesis where the failure of a given stage to proceed to completion can lead to the formation of side products thereby complicating what is otherwise a relatively straightforward separation procedure.

Although both invasive and non-invasive analytical methods are known for determining the products of solid phase synthesis (see *The Combinatorial Index, idem*), one of the recognised problems with solid phase synthesis is that it is generally more difficult to monitor the progress of a reaction; see for example the Czarnik paper referred to above. This is particularly true with the products of combinatorial chemistry methods where there is a need to use rapid high throughput techniques to analyse the large numbers of compounds generated by such methods. Techniques such as mass spectrometry are potentially ideally suited as a means of providing high throughput analyses, but a substantial problem is that not all compounds produce a guaranteed response under mass spectrometric conditions. Indeed, many compounds are "invisible" to the so-called "soft" methods of mass spectrometry such as Matrix-Assisted Laser-Desorption Ionisation (MALDI) and Electrospray mass spectrometry which are intended to detect molecular ions without causing significant fragmentation of the molecule.(in this context, the term "sfot " menas mthods of ms that give mol ions not fragments) One of the reasons for this is that under MALDI and

electrospray conditions, many compounds, particularly peptides, do not ionise sufficiently well to give a detectable mass spectral response.

One method which addresses this problem is disclosed in Carrasco *et al. Tetrahedron Letters*, 1997, 38, No. 36, pp 6331-6334. Carrasco's method involves the formation of a construct comprising a resin bead linked via a first linker group to a group which the authors refer to as an "ionisation tag". The "ionisation tag" in turn is linked via a second linker group to a substrate. The first and second linker groups are orthogonally cleavable, i.e. they can be selectively cleaved using different chemistries. In the specific example disclosed in Carrasco *et al.*, the first linker group is photochemically cleavable whilst the second linker group is chemically cleavable. The "ionisation tag" used by Carrasco is a tetrapeptide chain having the sequence (reading from the C-terminus) Gly-Phe-Lys-Ala, and having an N-(2-trimethylammonium)-acetyl group linked to the lysine. The purpose of the ionisation tag is twofold. Firstly, it provides an already ionised "sensitiser group" which enables the construct to be detected by matrix-assisted laser-desorption ionisation (MALDI) mass spectrometry,and secondly, it adds mass to the construct thereby enabling substrate molecules to be detected without being swamped or masked by low molecular weight peaks in the mass spectrum.

However, there are several significant potential problems with the Carrasco constructs and in particular the "ionisation tag" used in the construct. More particularly, it is envisaged that the quaternary ammonium sensitiser group will be incompatible with many of the types of chemistry that the chemist might wish to perform on the constructs. Thus, for example, the trimethylammonium group is potentially chemically reactive and, furthermore, the cationic group may function as an ion exchange centre and complex with anions.

It is an object of the present invention to provide a means of monitoring the progress of a chemical reaction on a solid support which avoids problems inherent in known methods and which provides a m ans of detecting and

identifying the products of solid phase synthetic techniques using mass spectroscopy.

Accordingly, in a first aspect, the invention provides a chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr comprising the substrate and at least a portion of the connecting group Y; characterised in that cleavage at the first cleavage site forms or introduces on the chemical fragment Fr at the first cleavage site a moiety comprising a sensitising group G which sensitises the chemical fragment Fr to instrumental, e.g. mass spectroscopic analysis.

In the constructs of the invention, the sensitising group G is formed or introduced by cleavage of the "skeleton" of the construct, and not by cleavage of a side chain or removal of a protecting group from a pendant sensitising group.

The sensitising group G renders the fragment Fr, and hence indirectly the substrate R, more sensitive to analysis by a given analytical technique, and in particular a mass spectrometric technique. Thus the sensitising group can be a group which is readily ionisable under the conditions encountered in a mass spectrometer, and in particular electrospray mass spectrometry, to afford a strong signal.

The ionisable sensitising group formed during the selective cleavage from the solid support of the ch mical fragment Fr serves to ensure that the fragment is ionised sufficiently in th mass sp ctrometer to give a strong r sponse. This overcomes a problem inherent in many molecules synthesised by solid phase

methods where a suitable ionising group is not present and analysis by high throughput mass spectral techniques is problematical.

The ionisable group can be for example a basic amino group or a carboxylate group but preferably it is a basic amino group. It will be appreciated that the term "basic amino group" as used herein refers in particular to an amino group which is readily protonated.

The basic amino group can be a primary amino group, a secondary amino group, or a tertiary amino group. Where the basic amino group is a tertiary amino group, it can be for example, a cyclic amino group such as piperidino, piperazino, pyrrolidino, or morpholino, piperidino or piperazino (e.g.N-methylpiperazino) being presently preferred.

In accordance with the invention, a moiety comprising the sensitising group G, such as a basic amino group, is formed or introduced at the first cleavage site as the chemical fragment Fr (hereinafter referred to as the analytical fragment) is cleaved from the solid support. The advantage of forming the sensitising group G in this way is that it avoids the potential problem of a pre-existing or preformed sensitising group interfering with the chemistry of the construct. Moreover, it avoids the need to have the sensitising group independently protected thereby obviating the additional problem of deprotection, a requirement which could limit the the types of chemistry available to the chemist for cleavage at the first and second cleavage sites. Thus, for example, if a protected pendant sensitising group were present, and for example the conditions required for deprotection involved the use of an acid such as trifluoroacetic acid, then this would effectively prevent the use of an acid labile cleavage site as the second cleavage site linking the substrate to the rest of the construct. The constructs of the invention avoid such problems.

The atoms or functional group making up the sensitising group G can be present in a masked form in the construct before cleavage of the fragment Fr

from the resin, the cleavage conditions merely serving to unmask the sensitising group G. Alternatively, the atoms or functional group making up or containing the sensitising group G can be introduced at the cleavage site during the cleavage reaction. For example, where the sensitising group is a basic amino group, the nitrogen atom of the amino group can be present in the construct before cleavage, or it can be introduced during the cleavage reaction.

When the sensitising group G is introduced from an external source during the cleavage reaction, it can form part of a larger chemical moiety. For example, the group G can be introduced as part of a group X-G wherein X is the residue of a nucleophile or electrophile, for example a nitrogen or sulphur-based nucleophilic group, e.g. a group of the formula G-Alk-Nuc wherein Alk is an alkylene group, and Nuc is a nucleophile, such as an amine or a thiolate group.

It is preferred that the chemical fragment Fr contains a means for imparting a characteristic signature to the mass spectrum of the fragment. The signature can advantageously be provided by incorporating into the fragment a "peak splitting" isotopic label. The peak splitting isotopic label comprises at least one atom that exists in a number of stable isotopic forms. By isotopic labelling of one or more particular atoms in the fragment Fr such that a given atom is labelled with a mixture of isotopes, the mass spectra of the molecular ions will appear as a characteristic pattern, the precise pattern depending on the relative amounts of the individual isotopes. Thus, for example, if a given atom in the fragment Fr is labelled such that 50% of the atoms are of one isotopic form and 50% are of another isotopic form, the mass spectrum will show the molecular ion as a characteristic doublet in which the peaks are of approximately equal height.

The purpose of the peak splitting atom(s) is to provide a characteristic pattern which will characterise any peak in the mass spectrum originating from the analytical fragment Fr, thereby distinguishing such peaks from those due to extraneous materials.

Examples of atoms that can be used as isotopic peak splitting labels include $^{1}H/H^{2}$ (D), $^{79}Br/^{81}Br$, $^{12}C/^{13}C$, $^{14}N/^{15}N$ and $^{16}O/^{18}O$.

The fragment Fr can contain a single peak splitting isotopic label or more than one such label. For example, the isotopic label can be a single bromine atom in which case the peak for the molecular ion of the analytical fragment Fr liberated following cleavage from the solid support will appear as a doublet. By introducing a second or subsequent peak splitting label(s), a more complex peak pattern will be produced for the molecular ion.

The isotopic peak splitting label(s) preferably is/are located between the first and second cleavage sites.

The first and second cleavage sites can be defined by first and second linker groups L^1 and L^2. A "spacer group" A can be interposed between the two linker groups L^1 and L^2, the spacer group A typically containing an isotopic peak splitting label. Accordingly, in one preferred embodiment of the invention, there is provided a chemical construct as hereinbefore defined wherein the connecting group Y has the formula L^1-A-L^2; wherein L^1 is a first linker group defining the first cleavage site; A is a chemical group (the spacer group) containing a peak splitting isotopic label; and L^2 is a second linker group defining the second cleavage site.

The first and second cleavage sites are orthogonally and selectively cleavable; i.e. the conditions used to effect cleavage at one of the cleavage sites will not cleave the other. A wide variety of different types of cleavage reaction can be used, examples being reactions selected from acid catalysed cleavage, base catalysed cleavage, oxidative cleavage, reductive cleavage, nucleophilic displacement, electrophilic displacement, and thermal, photochemical and enzymatic cleavage.

Thus, in a preferred embodiment, there is provided a chemical construct as hereinbefore defined wherein the first cleavage site is selectively cleavable by one type of chemistry selected from a group of chemistries consisting of cleavage under acid conditions, base catalysed cleavage, oxidative cleavage, reductive cleavage, nucleophilic displacement, electrophilic displacement, and thermal, photochemical and enzymic cleavage, and the second cleavage site is selectively cleavable by a different type of chemistry selected from the said group.

In one particularly preferred embodiment, the first cleavage site is cleavable under photochemical conditions or by oxidation followed by a nucleophilic displacement, and the second cleavage site is cleavable under acid conditions.

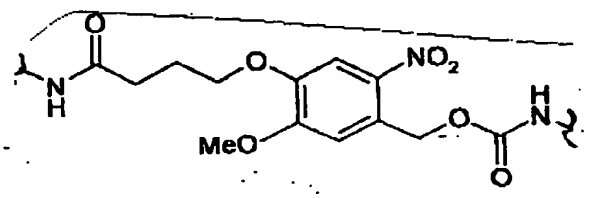
Either the first or second cleavage sites/linkers, or both, can be of the "safety catch" variety; i.e. the cleavage site or linker group must be chemically modified in a first step before it can be subjected to cleavage in a second step. An advantage of such an arrangement is that it prevents or significantly reduces the possibility of cleavage taking place inadvertently. One example of a "safety catch" mechanism involves oxidation of a functional group in a first step, the oxidation serving to make the functional group more amenable to displacement by a nucleophile in a subsequent cleavage step. The nucleophile can vary considerably in structure. For example, in one embodiment, the nucleophile can be an amino group-containing nucleophile), the amino group participating in the nucleophilic displacement action such that the amino group is attached directly to the cleavage site. Alternatively, in another embodiment, the amino group (or other sensitising group) can be present in a group (e.g. a dialkylaminoalkyl-thiolate anion) containing another nucleophile such as a sulphur nucleophile which becomes attached to the cleavage site.

Examples of linkers that can be selectively cleaved under acidic conditions include appropriately substituted benzyloxycarbonyl groups and

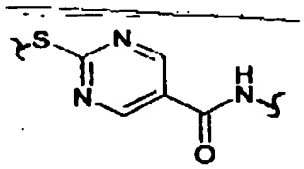
appropriately substituted diphenyl-methylamino groups, both of which can be cleaved by the action of trifluoroacetic acid.

Particular examples of acid cleavable linker groups are set forth in *The Combinatorial Index*, Barry A. Bunin, Academic Press, San Diego 1998, the disclosure of which is incorporated herein by reference. Linkers of the "Rink" or "Knorr" type typically comprise an N-protected 1-amino-1,1-diphenylmethane moiety, the amino group when deprotected allowing attachment to a substrate, one of the phenyl rings being substituted for example with dimethoxy groups and the other having a carboxyalkyloxy substituent providing a second point of attachment. Cleavage with TFA gives rise to a substrate compound having a terminal carboxamido group. Linkers of the "Wang" type typically contain a substituted phenoxyacetyl group, the acetyl group providing one point of attachment, and a benzylic hydroxyl group on the phenyl ring forming a second point of attachment. Esters can be formed between a carboxyl group of a substrate and the benzylic hydroxyl group, the ester groups being subsequently cleavable with TFA to release a substrate compound having a terminal carboxylate group.

Examples of groups that can be selectively cleaved under photochemical conditions include carbamate groups such as *o*-nitrobenzyloxycarbamate groups in which initial cleavage takes place between the benzylic methlene group and the adjacent oxygen atom, followed by loss of carbon dioxide to give a basic amino group. A particular example of a group that can be selectively cleaved under photochemical conditions is as follows:



Examples of groups that can be cleaved by nucleophilic displacement include mercaptopyrimidine-based "safety catch" linkers such as 5-carboxy-2-mercaptopyrimidine, where cleavage can be effected by reacting under oxidising conditions to generate a sulphoxide or sulphone linkage, followed by reaction with a nucleophilic amino group to form a 2-aminopyrimidine. A particular example of such a group is the mercaptopyrimidine shown below, which can be cleaved by reacting with a cyclic amine such as piperidine or an N-substituted piperazine (e.g.N-methylpiperazine), or an amino group-containing thiolate nucleophile (e.g. dimethylaminoethylthiolate) after first oxidising with an oxidising agent such as a per-acid, e.g. a perbenzoic acid such as *m*-chloroperbenzoic acid.



By making the first and second cleavage sites, e.g.as defined by the first and second linker groups L^1, L^2, orthogonally cleavable, it is possible selectively to separate from the construct either the chemical fragment Fr or the substrate R, simply by using different cleavage conditions. This means that during experiments designed to optimise the conditions for a particular reaction step, the chemist can subject the construct to conditions suitable for cleaving off the analytical fragment Fr thereby allowing analysis to be carried out to determine the outcome of each test reaction. Similarly, during a preparative reaction (e.g. preparation of a comiantorial librry or subsequent preparative reactions such as scale-up reaction or commercial production), quality control (QC) can be carried out by removing a number of solid supports from the reaction vessel, cleaving the constructs at the first cleavage site and analysing the resulting fragment Fr to s e whether a particular reaction step has worked. On the oth r hand, by cleaving at the second cleavage site e.g. on th second linker group, rath r than the first, the reaction product R is released from the solid support. Thus, an

advantage of the constructs of the present invention is that they can be used both at an experimental level to optimise a particular process step, and also at a preparative level without modifying the linker groups.

In one embodiment of the invention, the fragment Fr, and more preferably the spacer group A contains an alkylene diamine group or aminoalkoxy group. The precise size of the alkylene group and its degree of substitution is not currently considered to be important, but by way of example the chain could be from 2 to 30 carbon atoms in length, for example up to 20 carbon atoms, more typically less than 10 carbon atoms, particular examples being ethylene or propylene diamine or aminoalcohol groups which may be substituted or unsubstituted. The alkylene diamine group or aminoalcohol typically contains a peak splitting isotopic label as hereinbefore defined. The two amino groups can each be bonded to respectively the first and second linker groups. In order to increase the mass of the spacer group A, the alkylene diamine group can be substituted by an aryl group, or example an N-aryl group such as an N-benzyl group. The N-aryl group may optionally be substituted with one or more substituent groups.

Where, as is preferred, the spacer group contains one or more mass-spectral peak splitting isotopic labels, these can be located either in the alkylene chain or in a substituent group attached to the alkylene chain. Thus, for example, an N-benzyl group bonded to one of the two amino groups in an alkylenediamine can have a methylene group which is substituted with the peak splitting atom deuterium. Alternatively, an aryl ring (e.g. an N-benzyl group) present in a substituent on the alkylene diamine can be substituted with a peak splitting bromine atom, one particular example of an aryl group being an N-*o*-bromobenzyl group.

The chemical fragment Fr, and in particular the spacer group A, as well as providing a means of identification using mass spectrometry, can also be provided with one or more additional sensitisers to allow characterisation by

other analytical techniques. For example, the spacer group can contain a chromophore to allow characterisation by ultra violet (u.v.) or fluorescence spectroscopy.

Although alkylene diamine and amino alcohol groups are given as specific examples of spacer groups, alternative spacer groups can be used. For example, the spacer groups can be formed from hydrocarbon chains containing up to thirty or more carbon atoms in the chain which can be optionally interrupted with one or more heteroatoms such as oxygen, nitrogen or sulphur. As a further alternative, the spacer can be for example a peptide chain containing one or more amino acids. The precise nature and length of the spacer is not currently considered to be important provided that the spacer does not interfere with the chemistry of the construct.

The solid support Q can be any type of solid support suitable for use in solid phase synthesis, and in particular combinatorial chemistry. Thus, purely by way of example, the solid support can take the form of beads, a solid surface, solid substrates, particles, pellets, discs, capillaries, hollow fibres, needles, solid fibres, or organic or inorganic gels such as silica gels, and insoluble organic particles such as particles formed from fullerenes.

Examples of beads are polymeric beads such as cellulose beads or resin beads, particular examples of materials from which resin beads can be prepared including functionalised polymer resins such as polystyrene resins, polyacrylamide resins and dimethylacrylamide resins. Examples of suitable supports are listed in *The Combinatorial Index* by Barry A. Bunin, referred to above.

In another aspect, the invention provides a method of analysing the constructs hereinbefore defined; the method comprising cleaving the construct at the first cleavage site to release the chemical fragment Fr, the cleavage reaction generating on the chemical fragment Fr a mass spectrometric

sensitising group (e.g. a group which is ionisable under mass spectroscopic conditions), and then subjecting the chemical fragment to mass spectrometry, e.g. electrospray mass spectrometry.

The analysis of the fragment Fr provides information on the reaction history of the construct. Thus, by mass spectrometric analysis, it can readily be determined whether or not the desired substrate has been formed in a given reaction sequence. Analysis of the fragment Fr can therefore be used not only to characterise the substrate or product of the solid phase reaction sequence, but also to follow the progress of the reactions.

In a further aspect, the invention provides an intermediate chemical construct for use preparing a chemical construct as hereinbefore defined, the intermediate construct having the formula Q-Y' wherein Y' is a reactive or protected form of the group Y, and Q and Y are as hereinbefore defined.

In a still further aspect, the invention provides an intermediate construct of the formula Q-L^1-A^P wherein Q and L^1 are as hereinbefore defined and A^P is a reactive or protected form of the spacer group A containing a peak splitting isotopic label. In a particular embodiment, the intermediate construct has the general formula Q-L^1-NH-Alk-NH-X^1 wherein Alk is an alkylene group and X^1 is hydrogen or an aralkyl group. The intermediate construct is preferably isotopically labelled with a peak splitting combination of atoms such as $^1H/H^2$ (D), $^{79}Br/^{81}Br$, $^{12}C/^{13}C$, $^{14}N/^{15}N$ and $^{16}O/^{18}O$.

The invention will now be illustrated but not limited by reference to the following examples.

In the Examples, the following abbreviations are used.

Abbreviations

AcOH	Acetic acid
DMF	Dimethylformamide

DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DIC	Diisopropylcarbodiimide
TFA	Trifluoroacetic acid
DIPEA	Diisopropylethylamine
HATU	2-(1H-9-Azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
DMSO	Dimethylsulfoxide
PyBOP®	Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphoniumhexafluorophosphate
HOBT	N-Hydroxybenzotriazole
AA	Amino acid

EXAMPLE 1

Preparation of a photocleavable construct

A photocleavable construct was prepared following the reaction scheme shown in Scheme 1.

Experimental For Scheme 1

In the experiments reported below, all washing was carried out fivefold with the quoted solvents.

TentaGel NH2 (Rapp Polymere) (Resin 1) (30 g, 12.0 mmol) was swelled with DMF and to this was added a solution of HOBT (9.3 g, 69.0 mmol), DIC (6.48 ml, 41.4 mmol) and 4-[4-(1-Hydroxyethyl)-2-methoxy-5-nitrophenoxy) butanoic acid (11.8 g, 39.4 mmol) in DMF (420 ml) and the mixture shaken for 16 h. The resin was then drained and washed with DMF followed by MeOH then DCM and finally MeOH. This was then dried in vacuo to give resin 2.

Resin 2 (3 g, 11.9 mmol) was treated with a solution of carbonyldiimidazole (19.3 g, 119 mmol) in DCM (400 ml) and the whole heated at 50 °C with slow stirring for 5 h. The resin was then drained and washed with DCM followed by Et_2O, DCM, Et_2O and dried in vacuo to give r sin 3.

Resin 3 (15 g, 4.35 mmol) was swelled with DMF (200 ml) and to this was added a solution of 1-benzyl-1-tert-butoxycarbonyl-1,2-diaminoethane (17) (2.71 g, 10.9 mmol) and 1-(dideuterobenzyl)-1-tert-butoxycarbonyl-1,2-diaminoethane (16) (2.71 g, 10.9 mmol) in DMF (20 ml) and the whole heated at 50 °C with slow stirring for 16 h. The resin was drained and washed with DMF followed by Et_2O, DCM, and Et_2O. The resin was then dried in vacuo. The resin was then treated with a 1:4 solution of aq.TFA (95%) and DCM and shaken for 30 min. The resin was then drained and washed with DCM followed by Et_2O, DCM, Et_2O and the resin was dried in vacuo. The resin was then shaken with 10% solution of DIPEA in DMF for 1h and then drained, the washing was then repeated as above to give resin 4.

A solution of HATU (0.96 g,2.52 mmol), DIPEA (0.89 ml, 5.04 mmol) and p-[(R,S)-aδ-[1-(9H-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxy-benzyl]-phenoxybutanoic acid (1.42 g, 2.52 mmol) in DMF (30 ml) was added to Resin 4 (3 g, 0.84 mmol) and the whole shaken for 16 h. The resin was then drained and washed with DMF, and DCM. A solution of 20% piperidine in DMF (30 ml) was added to the resin and the whole shaken for 1h. The resin was then drained and washed with DMF, DCM followed by Et_2O, DCM and Et_2O and the resin was dried in vacuo.

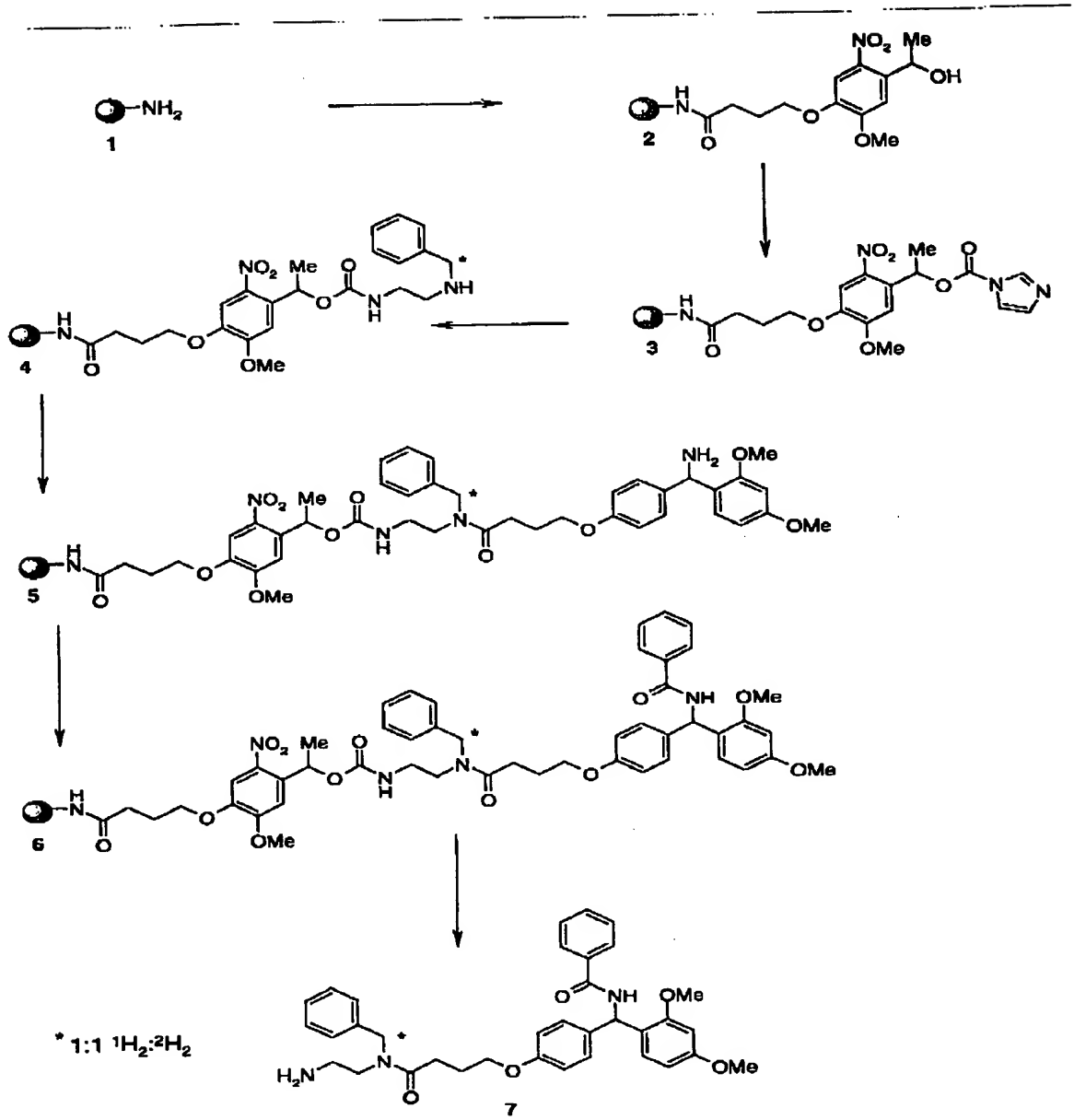
Resin 5 (20 mg, 0.004 mmol) was treated with a solution of HATU (15 mg, 0.04 mmol), DIPEA (14 mδl, 0.08 mmol) and benzoic acid (5 mg, 0.04 mmol) in DMF (1 ml) and the whole was shaken for 6 h. The resin was then drained and washed with DMF, DCM, Et_2O, DCM and Et_2O and dried in vacuo to give resin 6. Construct analysis of this resin by photocleavage followed by MS analysis is shown in figure 1 (see general cleavage conditions).

A small sample of the resin (ca. 0.5 mg) is suspended in DMSO (50 mδl) and exposed to UV light for 30 min. The solution is then analysed by mass

spectrometry. (Alternatively DMSO containing 0.2% hydrazine hydrate can be used as the solvent). Samples passed through a short capillary LC pre-column.

The mass spectra were obtained on a Finnegan MAT LCQ ion trap Mass Spectrometer operating in positive electrospray mode. Scan range 100 -1500 a.m.u. Scan cycle time 1.4 seconds; ion time 200 microseconds

Scheme 1


NH2
1
NO2 Me
OH
2
OMe
NO2 Me
O
O
N
N
3
OMe
NO2 Me
O
NH
4
OMe
NH2 OMe
5
OMe
O
NH OMe
6
OMe
NH OMe
H2N
7
OMe
* 1:1 1H2:2H2

EXAMPLE 2

Preparation of an oxidatively cleavable thiopyrimidine-containing construct

A construct containing an oxidatively cleavable thiopyrimidine linker group was prepared by the reaction scheme shown in Scheme 2 below.

Scheme 2

Experimental

A solution of 4-(chloromethyl)benzoic acid (0.27 g, 1.6 mmol), PyBOP® (0.83 g, 1.6 mmol) and DIPEA (0.56 ml, 3.2 mmol) in DMF (8 ml) was left to shook for 20 min then this solution was added to TentaGel NH2 (Rapp Polymere) resin 1 (1 g, 0.32 mmol) and the whole shaken for 5h. The resin was then drained and washed with DMF, DCM, Et_2O, DCM and finally Et_2O. The resin (8) was then dried in vacuo.

A slurry of thiourea (0.58 g, 7.7 mmol) in dioxane (4 ml) and ethanol (1 ml) was added to Resin 8 (1 g, 0.32 mmol) and the whole heated at 80 °C for 16h. The resin was drained and washed with DMF (x 5), DCM (x 5), Et_2O, DCM and finally Et_2O to give resin 9. The resin was then dried in vacuo.

A solution of methyl-2-(dimethylaminomethylene)-3-oxobutanoate (0.17 g, 0.96 mmol) and triethylamine (65 mδl, 0.48 mmol) in DMF (10 ml) was added to resin 9 (1 g, 0.31 mmol) and the whole heated at 80 °C for 16h. The resin was drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O. The resin (10) was then dried in vacuo.

Resin 10 (0.5 g, 0.15 mmol) was then treated with a 1:1 mixture of THF and 2N aq.NaOH (4 ml) and shaken for 2h. The resin was then drained and washed with THF/H2O, 10% AcOH/THF, THF/H2O, THF, DMF, DCM, Et_2O, DCM, Et_2O and then dried in vacuo to give resin 11.

A solution of PyBOP® (24 mg, 0.045 mmol) and DIPEA (16 mδl, 0.09 mmol) in DMF (1 ml) was added to resin 11 (50 mg, 0.015 mmol) followed by a solution of 1-tert-butoxycarbonyl-1-(o-bromobenzyl)-diaminoethane (18) (15 mg, 0.045

mmol) in DMF (1 ml) and the whole shaken for 3 days. The resin was drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O. A solution of 95% aq.TFA in DCM (20%, 1 ml) was then added to the resin and shaken for 2h. The resin was drained and washed with DMF, DCM, Et_2O, DCM, and then shaken with 10% DIPEA in DMF for 10 min. The resin was drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O. The resin (12) was then dried in vacuo.

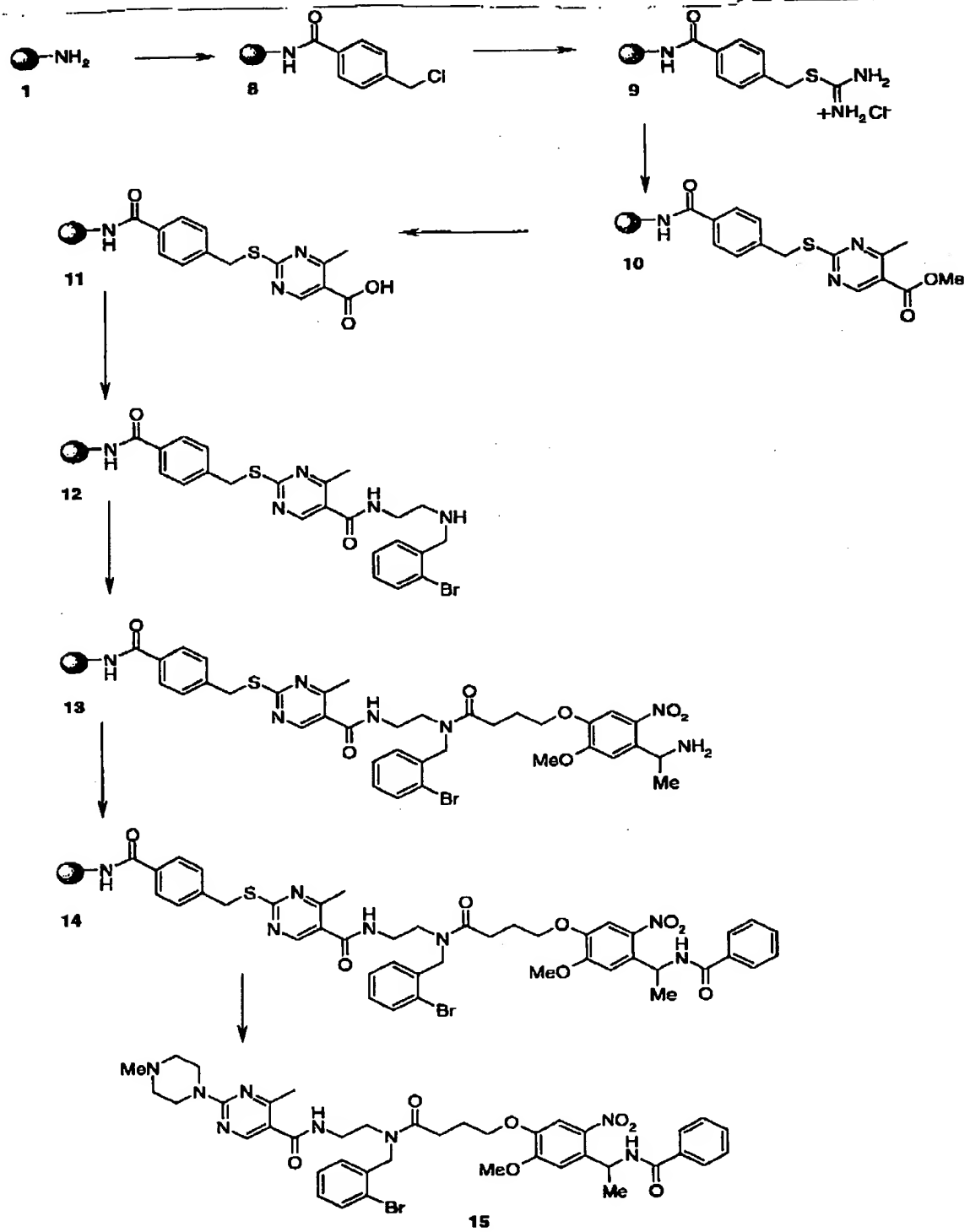
A solution of 4-[4-(1-(Fmoc-amino)ethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (23 mg, 0.045 mmol), DIPEA (16 mõl, 0.09 mmol) and HATU (17 mg, 0.045 mmol) in DMF (2 ml) was added to resin 12 (50 mg, 0.015 mmol) and the whole was shaken for 3h. The resin was drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O. The resin was then treated with 20% piperidine in DMF (3 ml) and the whole shaken for 1h. The resin (13) was then drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O and dried in vacuo.

A solution of benzoic acid (11 mg, 0.09 mmol), DIPEA (16 mõl, 0.09 mmol) and HATU (17 mg, 0.045 mmol) in DMF (2 ml) was added to resin 13 and the whole shaken for 2h. The resin (14) was then drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O.

The resulting construct can be subjected to analysis by pyrimidine cleavage conditions followed by MS analysis.

Thus, a small sample of resin (ca. 0.5 mg) is treated with 0.01M m-chloroperbenzoic acid (0.5 ml) for 2h. The resin is then drained and washed with DCM, Et_2O and DCM. The resin is then treated with 0.02 M N-methylpiperazine in DMF (50 mõl) is then added and shaken for 12h. The solution is then removed and analysed by mass spectrometry. The results of MS analysis of the construct ar shown in Figure 2.

Scheme 2



EXAMPLE 3

A number of different MS peak splitting groups have been prepared, and these are illustrated in Table 1 below.

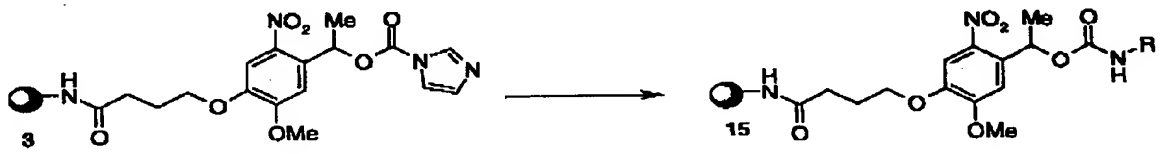


Table 1

In Example 1, the peak splitting group was introduced by means of the mixture of deuterium labelled and unlabelled compounds 16/17 in Table 1 (see reference to resin 4) whereas in Example 2, the peak splitting group was introduced by means of the bromo-benzyl compound 18 (see reference to resin 12).

Resins containing alternative peak-splitting groups were prepared using the compounds 19/20 and 21/22 as described below.

Use of amines 19/20

A solution of 1-tert-butoxycarbonyl-1,2-diaminoethane (20) (110 mg, 0.68 mmol) and doubly 15N labelled 1-tert-butoxycarbonyl-1,2-di(15N)aminoethane (19) (112 mg, 0.68 mmol) in DMF was added to resin 3 (0.6 g, 0.18 mmol) and the whole heated at 50 °C for 3h. The resin was then drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O. The resin was then dried in vacuo.

Use of amines 21/22

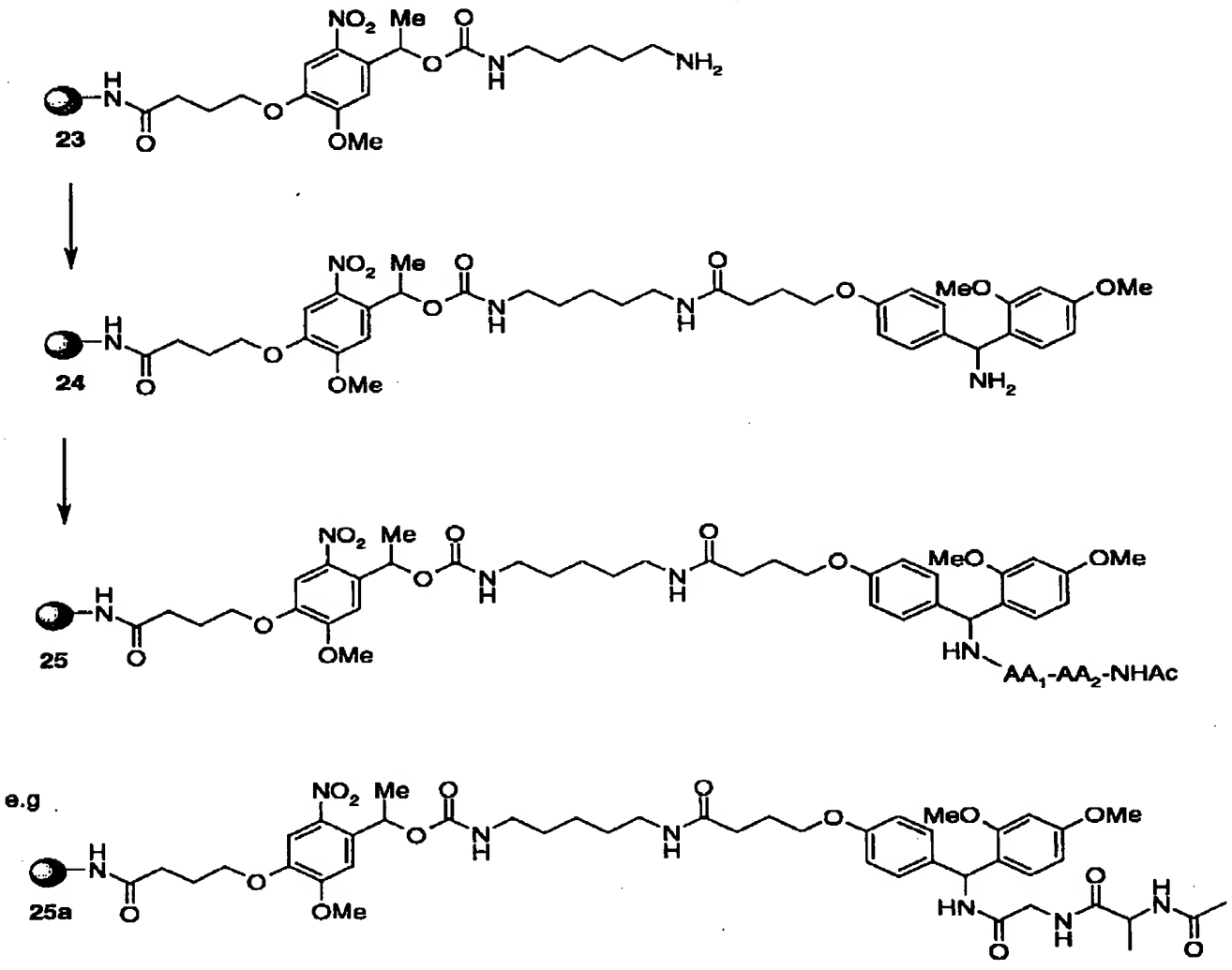
A solution of 1-tert-butoxycarbonyl-1,5-diaminopentane (21) (295 mg, 1.45 mmol) and doubly 15N labelled 1-tert-butoxycarbonyl-1,2-di(15N)aminopentane) (22) (295 mg, 1.45 mmol) in DMF was added to resin 3 (1.66 g, 0.58 mmol) and the whole heated at 50 °C for 4h. The resin was then drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O. A solution of 95% aq.TFA in DCM (20%, 1 ml) was then added to the resin and shaken for 2h. The resin was drained and washed with DMF, DCM, Et_2O, DCM, and then shaken with 10% DIPEA in DMF for 10 min. The resin (23) was then dried in vacuo.

EXAMPLE 4

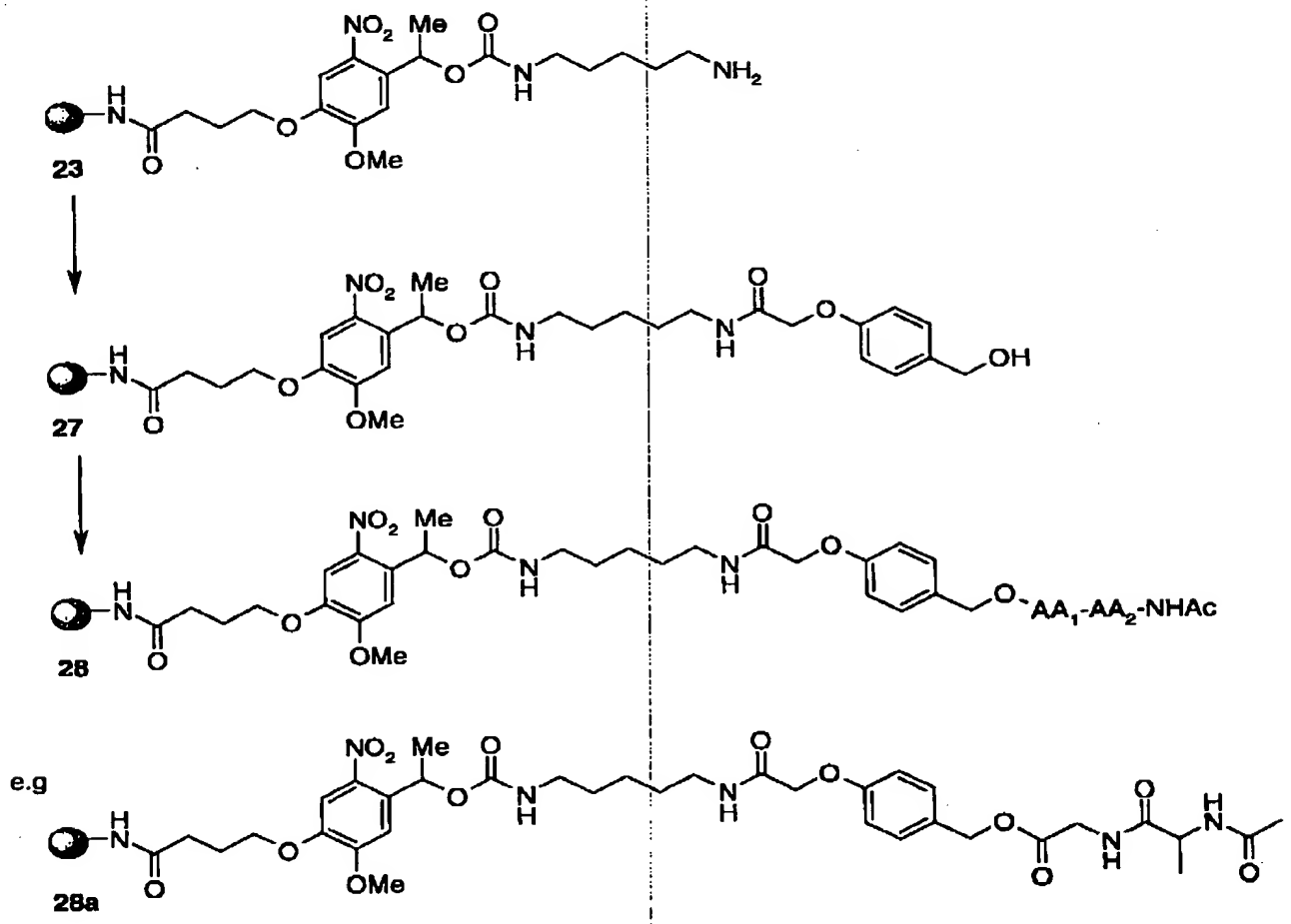
Preparation of Dipeptide-Containing Constructs

In Examples 1 and 2, the substrate or target compound in each case is the model compound benzamide. In the following Examples, constructs were prepared containing dipeptides, as the substrate, bound to either a Rink linker or a Wang linker as the second linker group. In each case, a photocleavable first linker group was used. The reaction schemes used to prepare the constructs are shown in Schemes 3 and 4 below.

Scheme 3



Scheme 4



Experimental for Amino acid examples

Preparation of Rink resin 24

A solution of p-[(R,S)-aδ-[1-(9H-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl]-phenoxybutanoic acid (240 mg, 0.42 mmol), DIC (65 mδl, 0.42 mmol) and HOBT (57 mg, 0.42 mmol) in DMF (1 ml) was added to resin 23 (100 mg, 0.042 mmol) and the whole shaken for 4h. The resin was then drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O. The resin was then treated with 20% piperidine in DMF (2 ml) and shaken for 30 min. The resin (24) was then drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O. The resin was then dried in vacuo.

Preparation of Wang resin 27

A solution of 4-formylphenoxyacetic acid (23 mg, 0.13 mmol), DIC (20 mδl, 0.13 mmol) and HOBT (17 mg, 0.13 mmol) in DMF (1 ml) was added to resin 23 (100 mg, 0.042 mmol) and the whole shaken for 4h. The resin was then drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O. The resin was then dried in vacuo. The resin was then treated with a solution of tetrabutylammonium borohydride (50 mg, 0.2 mmol) in DCM and shaken for 16h. The resin (27) was then drained and washed with DCM, DMF, DCM, Et_2O, DCM, and finally Et_2O.The resin was then dried in vacuo.

Preparation of acylated dipeptide sequence on resin

	RINK (25)			WANG (28)	
	AA1	AA2		AA1	AA2
Resin 25a	Gly	Ala	Resin 28a	Gly	Ala
Resin 25b	Val	Gly	Resin 28b	Val	Gly
Resin 25c	Ala	Val	Resin 28c	Ala	Val

Table 2: AA1 and AA2 on Rink and Wang linkers

The resins **25** and **28** were prepared by reacting the resins **24** and **27** respectively with the desired Fmoc protected amino acids followed by deprotection and then repeated coupling to the second aminoacid and deprotection (see general method). The terminal amino groups were then acylated using acetic anhydride (see method below).

Construct analysis of these resins by photocleavage followed by Electrospray MS analysis gave mass spectra as shown in the Figures listed in Table 3.

Analysed Resin	Figure No.
25a	3
25b	4
25c	5
28a	6
28b	7
28c	8

Table 3: Mass spectra after construct analysis

General/Typical Method for coupling the amino acids to the linkers

A solution of the Fmoc protected amino acid (0.14 mmol, 10 Eq), DIC (22 mõl, 0.14 mmol, 10 Eq) and HOBT (19 mg, 0.14 mmol, 10 Eq) in DMF (1ml) was added to required resins 24 and 27 (30 mg, ca. 0.014 mmol). The whole was then shaken for 5h. The resin was then drained and washed with DCM, DMF, DCM, Et_2O, DCM, and finally Et_2O. The Fmoc protecting groups were then be removed by the treatment of the resins with 20% piperidine solution in DMF (2 ml) and shaking for 30 min. The resin was then drained and washed with DCM, DMF, DCM, Et_2O, DCM, and finally Et_2O.The resin was then dried in vacuo.

Acetylation of the dipeptides

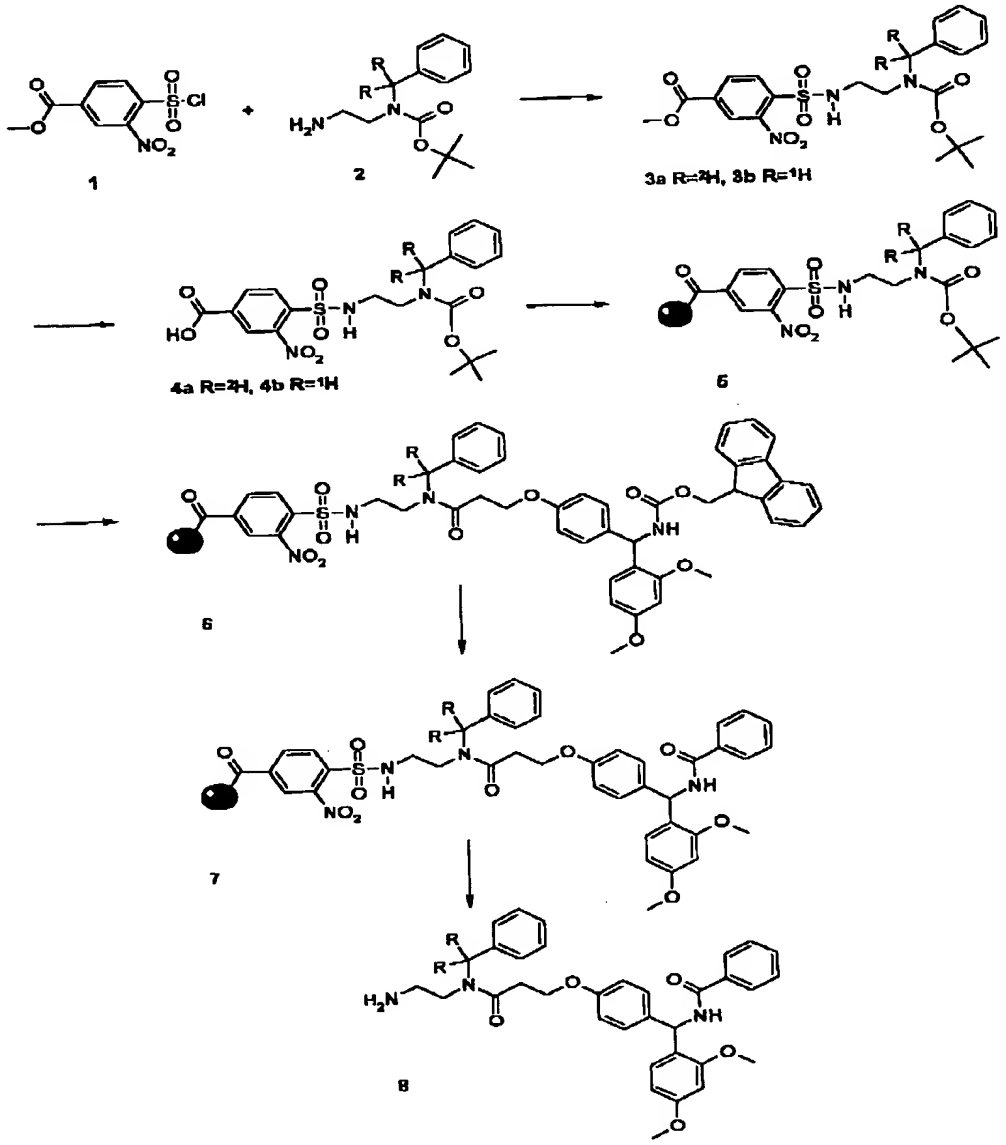
A solution of acetic anhydride (15 ml, 0.14 mmol) in DCM (1 ml) was added to the resins (30 mg, ca. 0.014 mmol) followed by DMAP (1 mg, 0.008 mmol) and the whole shaken for 2h. The resin was then drained and washed with DCM, DMF, DCM, Et_2O, DCM, and finally Et_2O. The resin was then dried in vacuo.

As can be seen from the above examples, the constructs of the invention enable small molecules formed by solid phase methods to be analysed readily and easily by mass spectrometry. By providing an ionisable group as a mass spectral sensitiser, and a peak splitting isotope, the result in each case has been a readily interpreted mass spectrum in which the molecular ion appears as a characteristic doublet, and in which peaks due to fragments or extraneous materials are negligible.

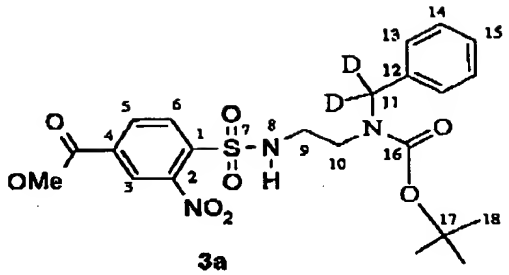
EXAMPLE 5

Preparation of sulphonamide-containing Construct

A construct containing benzoic acid as a model substrate linked via an acid labile "Rink" type linker to an ethylene diamine mass spectral peak splitting group and in turn via a sulphonamido linker to a resin was prepared as shown in the scheme below.



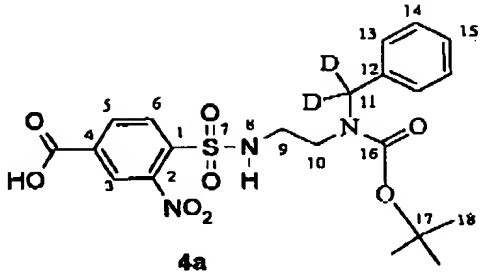
Preparation of Compound 3a



3a

To a stirred solution of 2-nitro-4-methylbenzoatesulfonyl chloride **1** (1.10 g, 3.90 mmol) and triethylamine (0.39 g, 3.90 mmol) in dry dichloromethane (40 ml), was added at 0°C the amine **2a** (1.00 g, 3.9 mmol) in dry dichloromethane (20 ml) over 30 min. The reaction mixture was stirred for a further 2 h at room temperature then concentrated *in vacuo* to give a yellow oil. Purification by chromatography with [MTBE:hexane (1:1)] as eluant followed by recrystallisation from MTBE (5 ml) and hexane (2 ml) furnished ***the sulfonamide 3a*** (1.53 g, 79%) as white flakes; mp 129-130°C; R_f 0.31 [MTBE:hexane (1:1)]; (Found: C, 53.2; H, 5.6; N, 8.5; S, 6.4. $C_{22}H_{25}D_2N_3O_8S$ requires C, 53.3; H, 5.5; N, 8.5; S, 6.5%); δ_H (DMSO-d^6) 1.40 (9H, s, H-18), 3.05 (2H, m, H-9), 3.20 (2H, dt, *J* 3 and 13, H-10), 3.9 (3H, s, O-Me), 7.10-7.35 (5H, m, H-13, 14 and 15), 8.09 (1H, d, *J* 8, H-6), 8.35 (1H, d, *J* 8, H-5), 8.40 (1H, m, H-8), 8.45 (1H, s, H-3).; δ_C (DMSO-d^6) 27.8 (C-18), 40.9 (C-9 and 10), 45.9 (C-11), 53.0 (O-Me), 79.1 (C-17), 124.8 (C-13), 127.0 (C-14 and 15), 128.3 (C-6), 130.0 (C-13), 132.9 (C-5), 134.2 (C-12), 136.4 (C-1), 147.5 (C-4), 154.7 (C-2), 163.5 (C-16 and C=O); MS (Electrospray +ve) m/z 496 $(MH)^+$; HPLC, R_t 6.07 min.

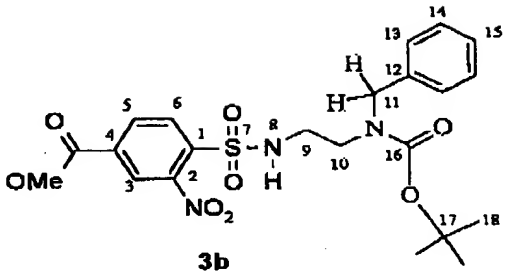
Preparation of Compound 4a



4a

The ester **3a** (0.40 g, 0.80 mmol) in methanol (2 ml) was treated with 1M aqueous sodium hydroxide (1.60 ml, 1.60 mmol) and stirred at room temperature for 1 hour. The reaction was then concentrated *in vacuo* and the residue dissolved in water (10 ml) then acidified with 1M hydrochloric acid (1.80 ml, 1.80 mmol) at 0°C. The resulting white precipitate was extracted with dichloromethane (3 x 10 ml). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure to ***the acid 4a*** (0.34 g, 88%) as a white solid; mp 143-145°C; R_F 0.58 [MTBE:hexane (1:1)]; δ_H (DMSO-d^6) 1.29 (9H, s, H-18), 3.02 (2H, m, H-9), 3.20 (2H, dt, *J* 3 and 13, H-10), 7.10-7.40 (5H, m, H-13, 14 and 15), 8.06 (1H, d, *J* 8, H-6), 8.28 (1H, s, H-3), 8.30 (1H, d, *J* 8, H-3); δ_C (DMSO-d^6) 27.9 (C-18), 40.9 (C-9 and 10), 45.9 (C-11), 79.1 (C-17), 124.5 (C-13), 127.1 (C-14 and 15), 128.4 (C-6), 129.5 (C-13), 132.5 (C-5), 139.2 (C-12), 147.3 (C-1), 155.1 (C-4), 164.8 (C-16 and C=O); (Found: MH^+, 482.156364 $C_{21}H_{23}D_2N_3O_8S$ requires MH+, 482.156615); HPLC, R_t 5.65 min.

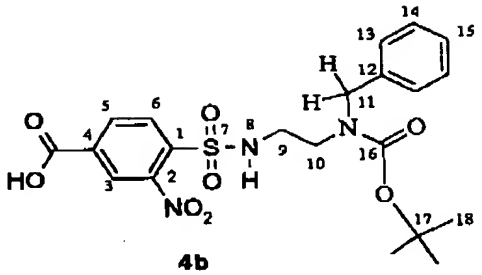
Preparation of Compound 3b



3b

To a stirred solution of 2-nitro-4-methylbenzoatesulfonyl chloride **1** (1.10 g, 3.90 mmol) and triethylamine (0.39 g, 3.90 mmol) in dry dichloromethane (40 ml), was added at 0°C the amine **2b** (0.95 g, 3.9 mmol) in dry dichloromethane (20 ml) over 30 min. The reaction mixture was stirred for a further 3 h at room temperature then concentrated *in vacuo* to give a yellow oil. Purification by chromatography with [MTBE:hexane (1:1)] as eluant followed by recrystallisation from MTBE (5 ml) and hexane (2 ml) furnished ***the sulfonamide 3b*** (1.51 g, 79%) as white flakes; mp 131-132°C; R_f 0.28 [MTBE:hexane (1:1)]; (Found: C, 53.7; H, 5.4; N, 8.4; S, 6.5. $C_{22}H_{27}N_3O_8S$ requires C, 53.5; H, 5.5; N, 8.5; S, 6.5%); δ_H (DMSO-d^6) 1.38 (9H, s, H-18), 3.10 (2H, t, *J* 8, H-9), 3.24 (2H, t, *J* 8, H-10), 3.95 (3H, s, O-Me), 4.32 (2H, s, H11), 7.13-7.32 (5H, m, H-13, 14 and 15), 8.09 (1H, d, *J* 8, H-6), 8.29 (1H, s, H-3), 8.32 (1H, d, *J* 8, H-3); δ_C (DMSO-d^6) 28.3 (C-18), 40.2 (C-9 and 10), 46.2 (C-11), 53.2 (O-Me), 79.3 (C-17), 125.1 (C-13), 127.2 (C-14 and 15), 128.6 (C-6), 130.3 (C-13), 133.1 (C-5), 134.4 (C-12), 136.6 (C-1), 147.6 (C-4), 163.7 (C-16 and C=O); MS (Electrospray -ve) m/z 492 $(M-H)^-$; HPLC, R_t 5.99 min.

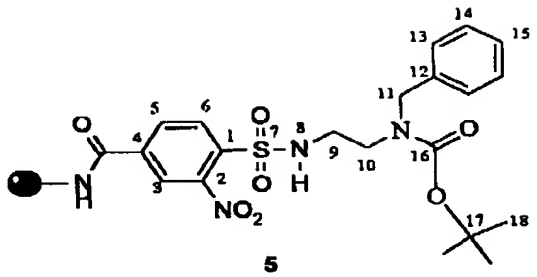
Preparation of Compound 4b



4b

The ester **3b** (0.40 g, 0.80 mmol) in methanol (2 ml) was treated with 1M aqueous sodium hydroxide (1.60 ml, 1.60 mmol) and stirred at room temperature for 1 hour. The reaction was then concentrated *in vacuo* and the residue dissolved in water (10 ml) then acidified with 1M hydrochloric acid (1.80 ml, 1.80 mmol) at 0°C. The resulting white precipitate was extracted with dichloromethane (3 x 10 ml). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure to ***the acid 4b*** (0.36 g, 93%) as a white solid; mp 123.7-124.8°C; R_F 0.59 [MTBE:hexane (1:1)]; δ_H (DMSO-d^6) 1.42 (9H, s, H-18), 3.12 (2H, m, H-9), 3.22 (2H, m, H-10), 4.38 (2H, s, H11), 7.18-7.42 (5H, m, H-13, 14 and 15), 8.11 (1H, d, *J* 8, H-6), 8.39 (1H, d, *J* 8, H-3); 8.45 (1H, s, H-3); δ_C (DMSO-d^6) 27.8 (C-18), 40.1 (C-9 and 10), 45.9 (C-11), 79.1 (C-17), 124.8 (C-13), 126.9 (C-14 and 15), 128.4 (C-6), 129.9 (C-13), 132.9 (C-5), 135.8 (C-12), 137.9 (C-1), 147.5 (C-4), 153.9 (C-2), 163.7 (C-16 and C=O); MS (Electrospray -ve) m/z 478 $(M-H)^-$; HPLC, R_t 5.56 min.

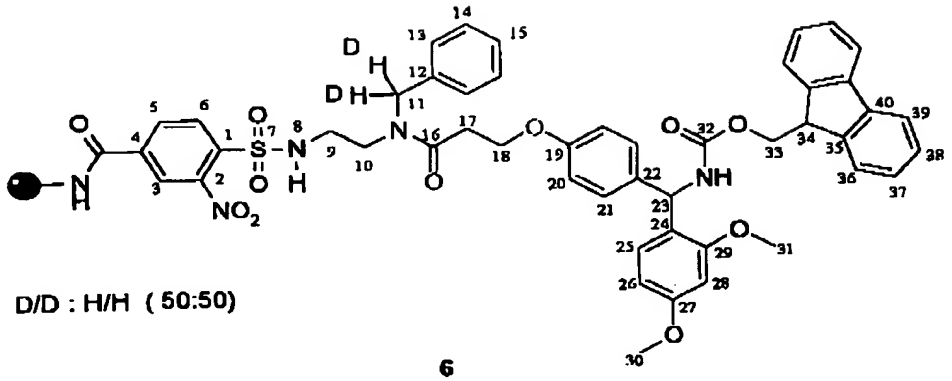
Preparation of Compound 5



5

To a suspension of amine resin (Argogel-NH_2) (0.37 g, 0.15 mmol) in DMF (4 ml) and dichloromethane (2 ml) was added the benzoic acid (**4a**) (0.22 g, 0.45 mmol), and the benzoic acid (**4b**) (0.22 g, 0.45 mmol), followed by PyBOP (0.47 g, 0.9 mmol) and HOBT (012. g, 0.90 mmol) followed, 4 min. later by Hünig's base (0.21 g, 1.80 mmol). The reaction was agitated with nitrogen for 18 h and the resin then washed with dichloromethane (6 x 10 ml), DMF (6 x 10 ml), dichloromethane (6 x 10 ml), ether (2 x 10 ml) and dried *in vacuo* to give the ***sulfonamide resin 5*** (0.43 g, 97%) as a yellow solid; Kaiser test was negative; Bromophenol Blue test was negative.

Preparation of Compound 6

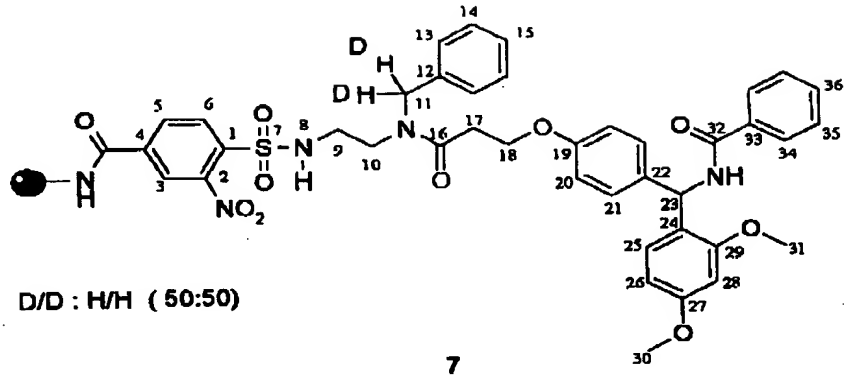


D/D : H/H (50:50)

6

The protected resin (0.39 g, 0.13 mmol) was suspended in dichloromethane (2 ml) and treated with trifluoroacetic acid (2 x 4 ml) for 5 min. Solvents were removed by filtration and the resin washed with 10% DIPEA solution in DMF (3 x 5 ml), dichloromethane (6 x 5 ml), DMF (6 x 5 ml), dichloromethane (6 x 5 ml), ether (2 x 5 ml). The amine resin was then suspended in DMF (4 ml) and dichloromethane (2 ml) the Rink acid (0.51 g, 0.90 mmol) was added, followed by PyBOP (0.47 g, 0.9 mmol) and HOBT (012. g, 0.90 mmol). After 4 min. Hünig's base (0.21 g, 1.80 mmol) was added. The reaction was agitated with nitrogen for 18 h and the resin then washed with dichloromethane (6 x 10 ml), DMF (6 x 10 ml), dichloromethane (6 x 10 ml), ether (2 x 10 ml) and dried *in vacuo* to give the ***sulfonamide resin 6*** (0.43 g, 100%) as a yellow solid; Kaiser test was negative; Bromophenol Blue test was negative; Resin loading 98% as estimated by quantitative Fmoc group cleavage.

Preparation of Compound 7

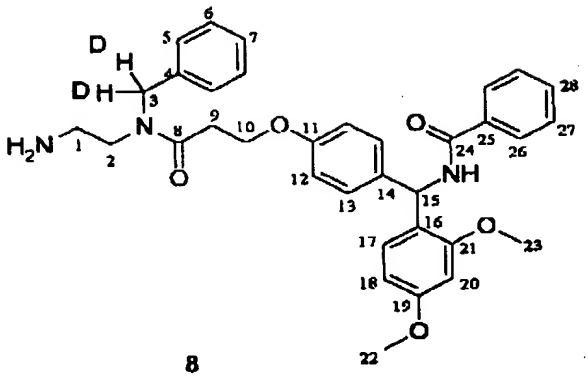


D/D : H/H (50:50)

7

Resin (6) (0.20 g, 0.06 mmol) was treated with 20% Piperidine in DMF solution (2 x 3 ml) for 10 min. Solvents were removed by filtration and the resin washed with DMF (6 x 3 ml), dichloromethane (6 x 3 ml), ether (2 x 3 ml). The resin was suspended in DMF (1 ml) and dichloromethane (1 ml) and benzoic acid (12 mg, 0.01 mmol) was added, followed by PyBOP (52 mg, 0.1 mmol) and HOBT (13 mg, 0.1 mmol). After 4 min. Hünig's base (26 mg, 0.2 mmol) was added. The reaction was agitated with nitrogen for 3 h and the resin then washed with dichloromethane (6 x 1 ml), DMF (6 x 1 ml), dichloromethane (6 x 1 ml), ether (2 x 1 ml) and dried *in vacuo* to give the ***sulfonamide resin 7*** (51 mg, 100%) as a yellow solid; Kaiser test was negative; Bromophenol Blue test was negative;

Preparation of Compound 8



D/D : H/H (50:50)

8

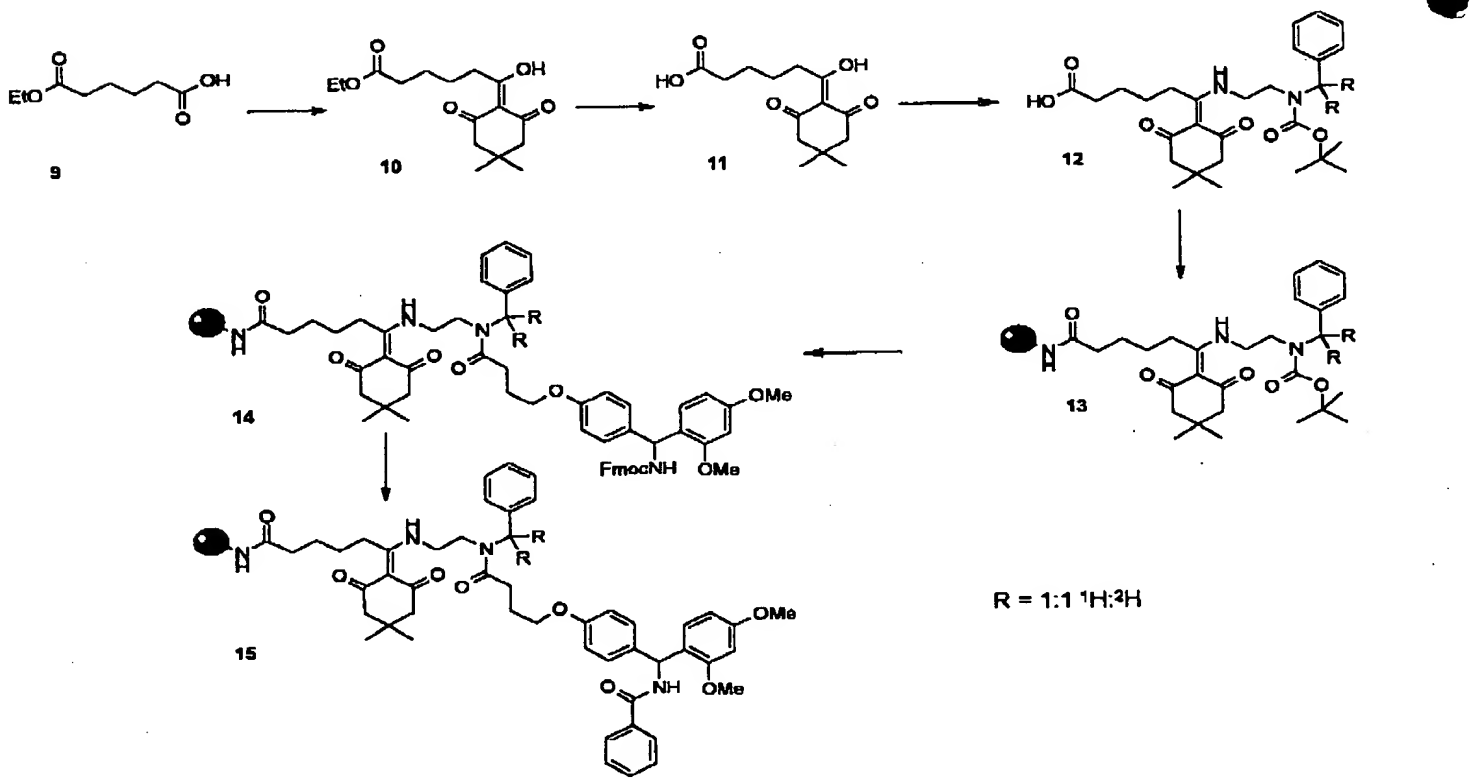
A small sample of resin (7) (ca. 0.5 mg) was treated with deprotecting solution A (0.5 ml) for 1 hr. The resin was filtered and the resulting solution was then analysed by electrosray mass spectrometry; MS (Electrospray -ve) m/z 582/584 $(M-H)^-$; HPLC, R_t 4.49 min.

Solution A: Mercaptoethanol (0.23 ml, 3.0 mmol) and DBU (0.67 ml, 4.5 mmol) in MeCN (3 ml).

EXAMPLE 5

Preparation of a dde-containing Construct

A construct containing a benzoic acid model substrate linked via a "Rink" type linker to an ethylene diamine mass spectral peak splitter and thence via a "dde" linker to a resin was prepared according to the reaction scheme set out below.

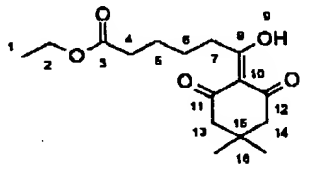


General

HPLC was run on a Hewlett Packard 1050 instrument using a C_{18} reverse phase column (Supelco, Supelcosil ABZ+, 3.3 mm, 4.6 mm ϕ). Method A: 10 to 95% solvent B gradient (1 ml/min) as the mobile phase. [Solvent A: 1% TFA in water; solvent B: 0.5% TFA in MeCN:water (10:1), 8 min gradient time], or

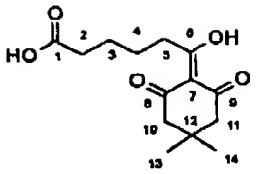
method B: C_{18} reverse phase column (Dynamax 60A, 25 mm, 6 mm ϕ) with 10 to 95% solvent B gradient (1 ml/min) as the mobile phase. [Solvent A: 1% TFA in water; Solvent B; 0.5% TFA in MeCN:water (10:1), 10 min gradient time]. Melting points were performed on a Mettler FP5 automatic melting point apparatus in open tubes heating from 50°C at 2°C min^{-1} and are uncorrected.

Preparation of Compound 10



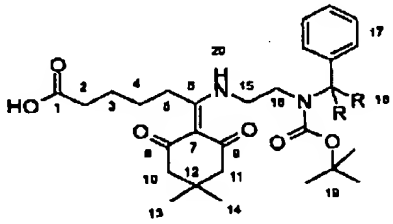
4-dimethylaminopyridine (4.03 g, 33 mmol) was added to a solution of adipic acid monoethylester (5.33 ml, 30 mmol) in dimethylformamide (50 ml) followed by diisopropylcarbodiimide (5.17 ml, 33 mmol). After 25 min, a solution of dimedone (4.63 g, 33 mmol) in dimethylformamide (25 ml) was added and stirring continued for 48 h. The reaction mixture was diluted with ethyl acetate (300 ml) and washed with 0.5M $KHSO_4$ (2 x 100 ml), water (100 ml) and saturated brine (50 ml), then dried ($MgSO_4$), filtered and evaporated. The resulting slurry was triturated with ethyl acetate (250 ml), filtered and the residue washed with ethyl acetate. Evaporation of the combined filtrates gave a yellow gum which was purified by solid phase extraction (1.5" x 4" column, eluant: dichloromethane) to give ***compound 10*** as a yellow oil (7.43 g, 84%). δ_H ($CDCl_3$, 400MHz): 4.04 (q, 2H, H_2, 7 Hz), 2.97 (t, 2H, H_4, 7.5 Hz), 2.46 (s, 2H, H_{13}), 2.28 (s, 2H, H_{14}), 2.26 (d, 2H, H_7, 7.5 Hz), 1.60 (m, 4H, $H_{5,6}$), 1.00 (s, 6H, H_{16}). δ_C ($CDCl_3$, 400 MHz): 205.15 (C-12), 197.58 (C-11), 195.08 (C-3), 173.47 (C-8), 112.02 (C-10), 60.27 (C-2), 52.67 (C-4), 46.80 (C-13), 40.04 (C-14), 34.15 (C-7), 30.72 (C-15), 28.23 (C-16), 24.64 (C-5), 24.01 (C-6), 14.31 (C-1). ν_{max} (cm^{-1}): 2960 (OH), 1734 (C=O ester), 1665 (C=O ketone), 1564, 1445, 1143. MS: (electrospray +ve) m/z 297 $(MH)^+$ (el ctrospray -ve), 295 $(M-H)^-$. HPLC method A: 5.31 min, 97%. 254nm.

Preparation of Compound 11



2M sodium hydroxide solution (2 ml, 4 mmol) was added to a solution of compound **10** in tetrahydrofuran (5 ml). After 3 h, the reaction mixture was poured onto 10% aq. hydrochloric acid (50 ml) and extracted with ethyl acetate (2 x 50 ml). The combined organic phases were dried ($MgSO_4$), filtered and evaporated to give ***compound 11*** as an off white solid (498.5 mg, 95%). δ_H ($CDCl_3$, 400MHz): 2.99 (dd, 2H, H_2, 5 Hz), 2.47 (s, 2H, H_{10}), 2.34 (dd, 2H, H_6, 5Hz), 2.29 (s, 2H, H_{11}), 1.63 (m, 4H, $H_{3,4}$), 1.00 (s, 6H, $H_{13,14}$). δ_C ($CDCl_3$, 400 MHz): 205.15 (C-9), 197.58 (C-8), 195.08 (C-1), 173.47 (C-6), 112.02 (C-7), 52.96 (C-2), 47.11 (C-10), 40.40 (C-11), 33.92 (C-5), 30.72 (C-12), 28.23 (C-13,14), 24.64 (C-3), 24.01 (C-4). ν_{max} (cm^{-1}): 3030 (acid OH), 1702 (acid C=O), 1646 (ketone C=O). Melting point: 58.2°C. MS: (electrospray +ve) m/z 269 $(MH)^+$ (electrospray -ve), 267 $(M-H)^-$. HPLC method A: 4.15 min, 97%. 254nm.

Preparation of Compound 12

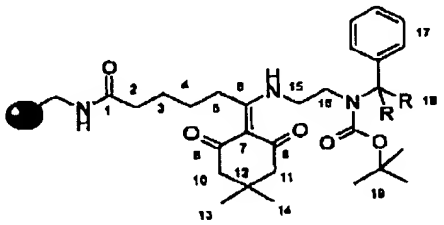


R = ^1H:^2H 1:1

A solution of *N*-(*tert*-butoxycarbonyl)-*N*-benzyldiaminoethane (187.3 mg, 0.75 mmol) and *N*-(*tert*-butoxycarbonyl)-*N*-(α-dideutero)benzyldiaminoethane (189.3 mg, 0.75 mmol) in dichloromethane (3 ml) followed by diisopropylethylamine (348.4 µl, 2 mmol) was added to a solution of compound **11** (268.4 mg, 1 mmol) in dichloromethane (2 ml). After stirring for 20 h, the reaction mixture was evaporated, redissolved in ethyl acetate (25 ml) and washed with 10%

citric acid solution (3 x 10 ml), water (10 ml) and saturated brine (10 ml). The organic phase was dried ($MgSO_4$), filtered and evaporated then purified by flash chromatography (1 to 3% methanol in chloroform) to give ***compound 12*** as a yellow gum (474.5mg, 95%). δ_H ($CDCl_3$, 400MHz): 13.50 (bs, 1H, H_{20}), 7.30 (m, 5H, H_{17}), 4.45 (bd, 1H, H_{18}), 3.47 (bd, 4H, $H_{15,16}$), 2.90 (m, 2H, H_2), 2.40 (t, 2H, H_5, 7.4Hz), 2.30 (s, 4H, $H_{10,11}$), 1.75 (m, 2H, H_4), 1.45 (m, 11H, $H_{3,19}$). ν_{max} (cm^{-1}): 3030 (acid OH), 1730 (C=O, Boc, acid), 1690 (ketone C=O), 1570 (conj. enamine). MS: (electrospray +ve) m/z 503, 501 $(MH)^+$ (electrospray -ve), 502, 499 $(M-H)^-$. HPLC method A: 5.35 min, >97%. 230, 254nm.

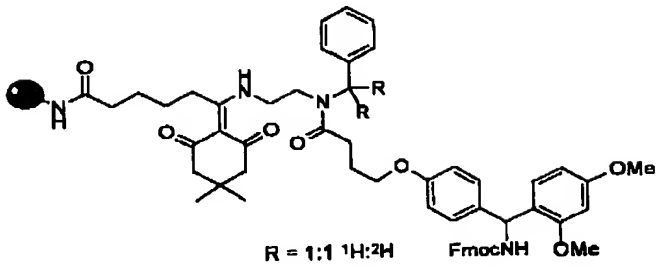
Preparation of Compound 13



R = ^1H:^2H 1:1

To a solution of compound 12 (310 mg, 0.62 mmol) in dichloromethane (6 ml) was added 1-hydroxybenzotriazole (110.8 mg, 0.82 mmol) and PyBOP (426.6 mg, 0.82 mmol). After 5 min, the solution was added to a suspension of aminomethyl Argogel® (500 mg, 0.205 mmol, 0.41 mmol/g) in dichloromethane (1 ml). The reaction mixture was agitated for 5 min then diisopropylethylamine (285.7µl, 1.64 mmol) was added and agitation continued for 16 h. The resin was washed with DMF (5 x) and DCM (5 x) then dried *in vacuo* to give ***resin 13*** (573.5 mg). The resin gave a negative bromophenol blue test.

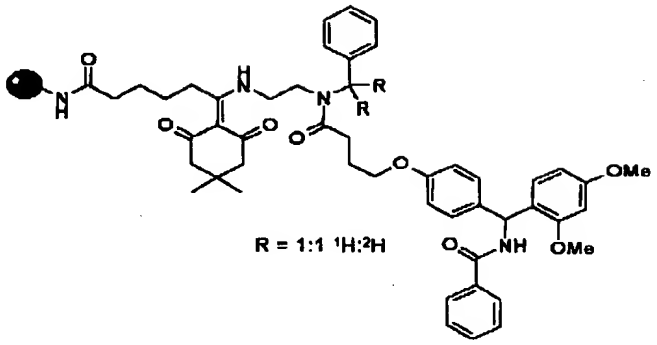
Preparation of Compound 14



Resin 13 (475.5 mg) was swollen with dichloromethane then treated with a solution of phenol (60 mg) in dichloromethane (1ml) followed by trifluoroacetic acid (3ml). The reaction mixture was agitated for 10 min then drained and washed with dichloromethane (2 x). The reaction was repeated then the resin washed with dichloromethane (1 x), 20% diisopropylethylamine in dimethylformamide (3 x), dimethylformamide (5 x) and dichloromethane (5 x) to give the amino resin intermediate which gave a positive bromophenol blue. The resin was suspended in dichloromethane (2 ml) and treated with a preformed solution of the C-4 RINK acid (351.5 mg, 0.62 mmol), 1-hydroxybenzotriazole (110.8 mg, 0.82 mmol) and PyBOP (426.6 mg, 0.82 mmol) in dimethylformamide (2 ml). After 5 min, diisopropylethylamine (285.7µl, 1.64 mmol) was added and the reaction mixture agitated for 3 h after which the resin gave a negative bromophenol blue test. The resin was washed with DMF (5 x) and DCM (5 x) then dried *in vacuo* to give ***resin 14*** (500.9 mg).

A small sample of resin 14 (ca. 0.5 mg) was treated with 2% hydrazine in dimethyl formamide for 30 minutes. The solution was then analysed by electrospray mass spectrometry to give an ion 700/702.

Preparation of Compound 15



Resin **14** (65 mg) was treated with 20% piperidine in dimethylformamide (2 x 3 ml x 10 min) then washed with dimethylformamide (5 x) and dichloromethane (5 x). The resin gave a positive Kaiser test and was suspended in dichloromethane (1 ml) then treated with a preformed solution of benzoic acid (127 mg, 1.04 mmol), 1-hydroxybenzotriazole (140 mg, 1.04 mmol) and PyBOP (541 mg, 1.04 mmol) in dimethylformamide (2 ml). After 5 min, diisopropylethylamine (541 mgμl, 2.08 mmol) was added and the reaction mixture agitated for 72 h after which the resin gave a negative Kaiser test. The resin was washed with DMF (5 x) and DCM (5 x) then dried *in vacuo* to give ***resin 15*** (60 mg).

A small sample of the resin (ca. 0.5mg) was treated with 2% hydrazine in dimethylformamide for 30 minutes. The solution was then analysed by electrospray mass spectrometry to an ion 582/584.

The foregoing examples are intended merely to illustrate the invention and should not be construed as imposing any limitations on the scope of the claims. It will readily be apparent that numerous alternations and modifications could be made to the constructs illustrated in the examples without departing from the principles underlying this invention and all such modifications and alterations are intended to be embraced by this application.

CLAIMS

1. A chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr comprising the substrate and at least a portion of the connecting group Y; characterised in that cleavage at the first cleavage site forms or introduces on the chemical fragment Fr at the first cleavage site a moiety comprising a sensitising group G which sensitises the chemical fragment Fr to instrumental, e.g. mass spectroscopic, analysis.

2. A chemical construct according to claim 1 wherein the chemical fragment Fr contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

3. A chemical construct according to claim 2 wherein the characteristic signature is provided by incorporating into the fragment Fr a peak splitting isotopic label.

4. A chemical construct according to claim 3 wherein the peak splitting isotopic label is defined one or more isotope pairs selected from $^{1}H/H^{2}$ (D), $^{79}Br/^{81}Br$, $^{12}C/^{13}C$, $^{14}N/^{15}N$ and $^{16}O/^{18}O$.

5. A chemical construct according to any one of claims 2 to 4 wherein the means for imparting a characteristic signature to the mass spectrum of the fragment is located between the first and second cleavage sites.

6. A chemical construct according to any one of the preceding claims wherein the first and second cleavage sites cleavage sites are defined by first and second linker groups L^1 and L^2.

7. A chemical construct according to claim 6 wherein an spacer group A is interposed between the two linker groups L^1 and L^2, the spacer group A containing means for imparting a characteristic signature to the mass spectrum of the fragment Fr as defined in any one of claims 2 to 5.

8. A chemical construct according to claim 7 wherein the wherein the connecting group Y has the formula L^1-A-L^2.

9. A chemical intermediate construct according to claim 8 wherein the group A has the general formula NH-Alk-NH-X^1 wherein X^1 is hydrogen or an aralkyl group, and Alk is an alkylene group.

10. A chemical construct according to any one of the preceding claims wherein the sensitising group G is an ionisable group which is ionisable under mass spectrometric conditions.

11. A chemical construct according to claim 10 wherein the group G is ionisable to form a positive ion under mass spectrometric conditions, for example electrospray mass spectrometric conditions.

12. A chemical construct according to any one of the preceding claims wherein the group G is a basic amino group.

13. A chemical construct according to claim 12 wherein the basic amino group is a primary amino group.

14. A chemical construct according to claim 12 wherein the basic amino group is a tertiary amino group.

15. A chemical construct according to claim 14 wherein the tertiary amino group is a cyclic amino group.

16. A chemical construct according to claim 15 wherein the cyclic amino group is N-methylpiperazino.

17. A chemical construct according to claim 12 or claim 13 wherein the basic amino group is derived from the photochemical cleavage of a carbamate group.

18. A chemical construct according to any one of claims 3 to 17 wherein the peak splitting isotopic label is contained within a substituted or unsubstituted alkylene diamine group.

19. A chemical construct according to claim 18 wherein the alkylene diamine group is substituted by an N-benzyl group.

20. A chemical construct according to claim 19 wherein the N-benzyl group has a methylene group which is substituted with the peak splitting atom deuterium.

21. A chemical construct according to any one of the preceding claims wherein the first cleavage site is selectively cleavable by one type of chemistry selected from a group of chemistries consisting of cleavage under acid conditions, base catalysed cleavage, oxidative cleavage, reductive cleavage, nucleophilic displacement, electrophilic displacement, and thermal, photochemical and enzymatic cleavage, and the second cleavage site is selectively cleavable by a different type of chemistry selected from the said group.

22. A chemical construct according to claim 21 wherein the first cleavage site is cleavable under photochemical conditions or by oxidation followed by a nucleophilic displacement, and the second cleavage site is cleavable under acid conditions.

23. A chemical construct according to claim 22 wherein the first cleavage site is cleaved by oxidation followed by nucleophilic displacement.

24. A chemical construct according to claim 23 wherein the nucleophile is an amine.

25. A chemical construct according to claim 24 wherein the amine is a cyclic amine such as piperidine.

26. A chemical construct according to any one of the preceding claims wherein one of the first and second cleavage sites is cleavable under acid conditions.

27. A chemical construct according to claim 26 wherein the acid cleavable cleavage site is cleavable by trifluoroacetic acid.

28. A chemical construct according to claim 26 or 27 wherein the acid cleavable cleavage site is the second linker group.

29. A method of analysing the constructs of any one of the preceding claims; the method comprising cleaving the construct at the first cleavage site to release the chemical fragment Fr, the cleavage reaction generating on the chemical fragment Fr at the cleavage site a group comprising a mass spectrometric sensitising group G (e.g. a group which is ionisable under mass spectrosc pic c nditions), and then subjecting the chemical fragment t mass spectrometry, e.g. electrospray mass spectrom try.

30. An intermediate chemical construct for use preparing a chemical construct as defined in any one of the preceding claims, the intermediate construct having the formula Q-Y' wherein Q' is a reactive or protected form of the group Q.

31. An intermediate construct of the formula $Q\text{-}L^1\text{-}A^P$ wherein Q and L^1 are as defined in any one of the preceding claims and A^P is a reactive or protected form of the spacer group A containing a peak splitting isotopic label.

32. An intermediate construct according to claim 31 having the general formula $Q\text{-}L^1\text{-}NH\text{-}Alk\text{-}NH\text{-}X^1$ wherein X^1 is hydrogen or an aralkyl group, and Alk is an alkylene group.

Figures
Figure 1 L981994

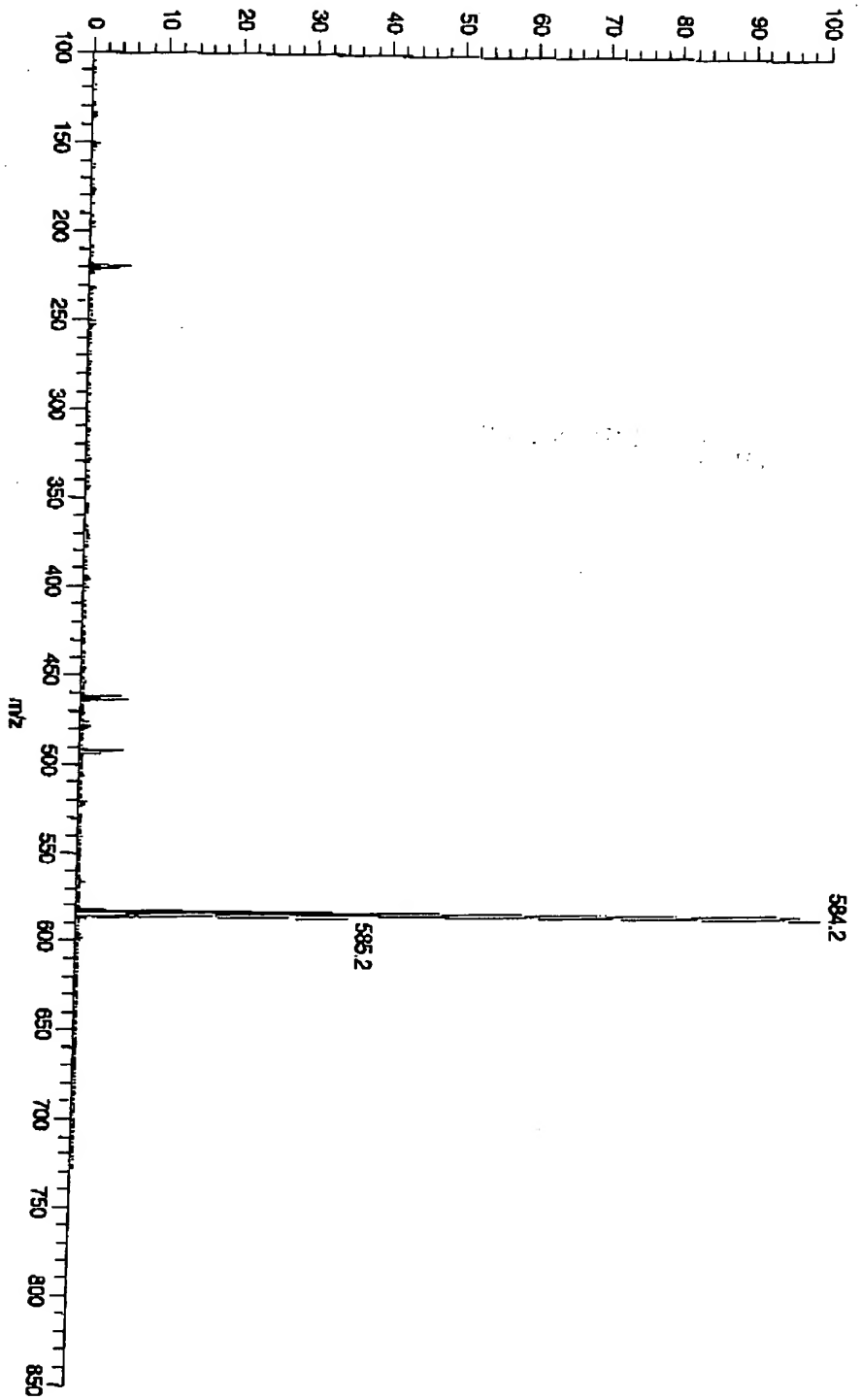


Figure 2 L985240

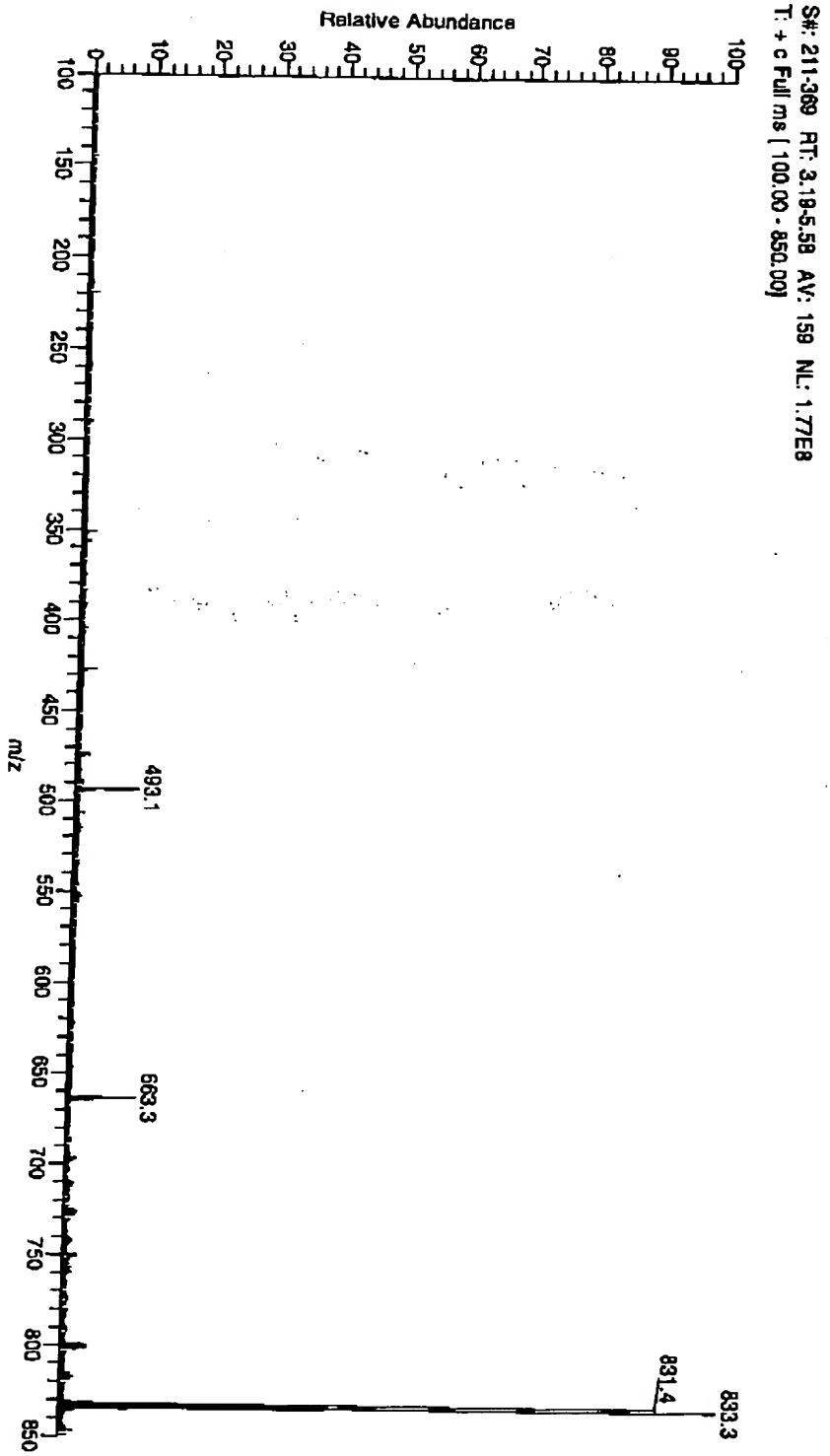


Figure 3 L981291

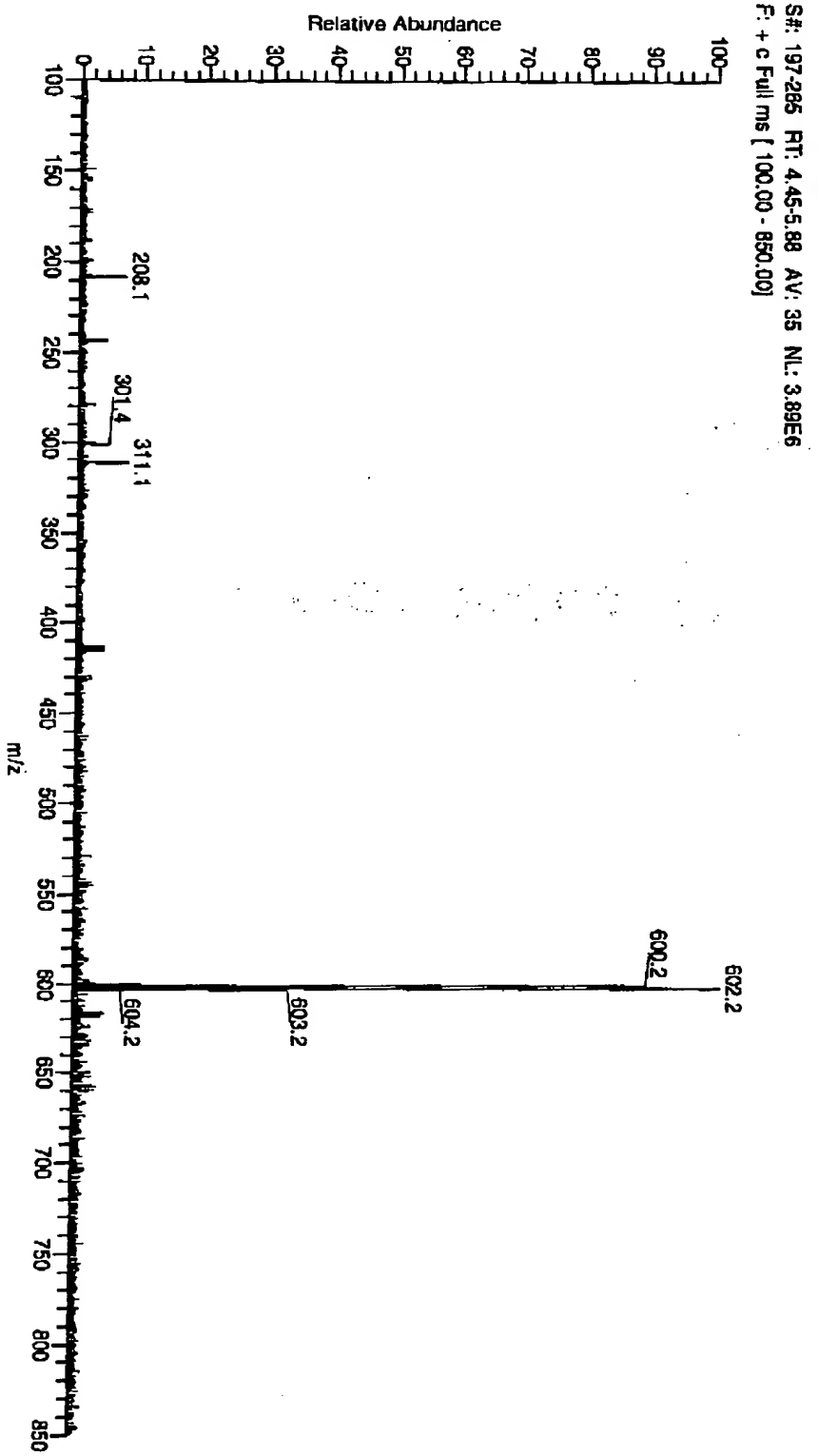


Figure 4 L981290

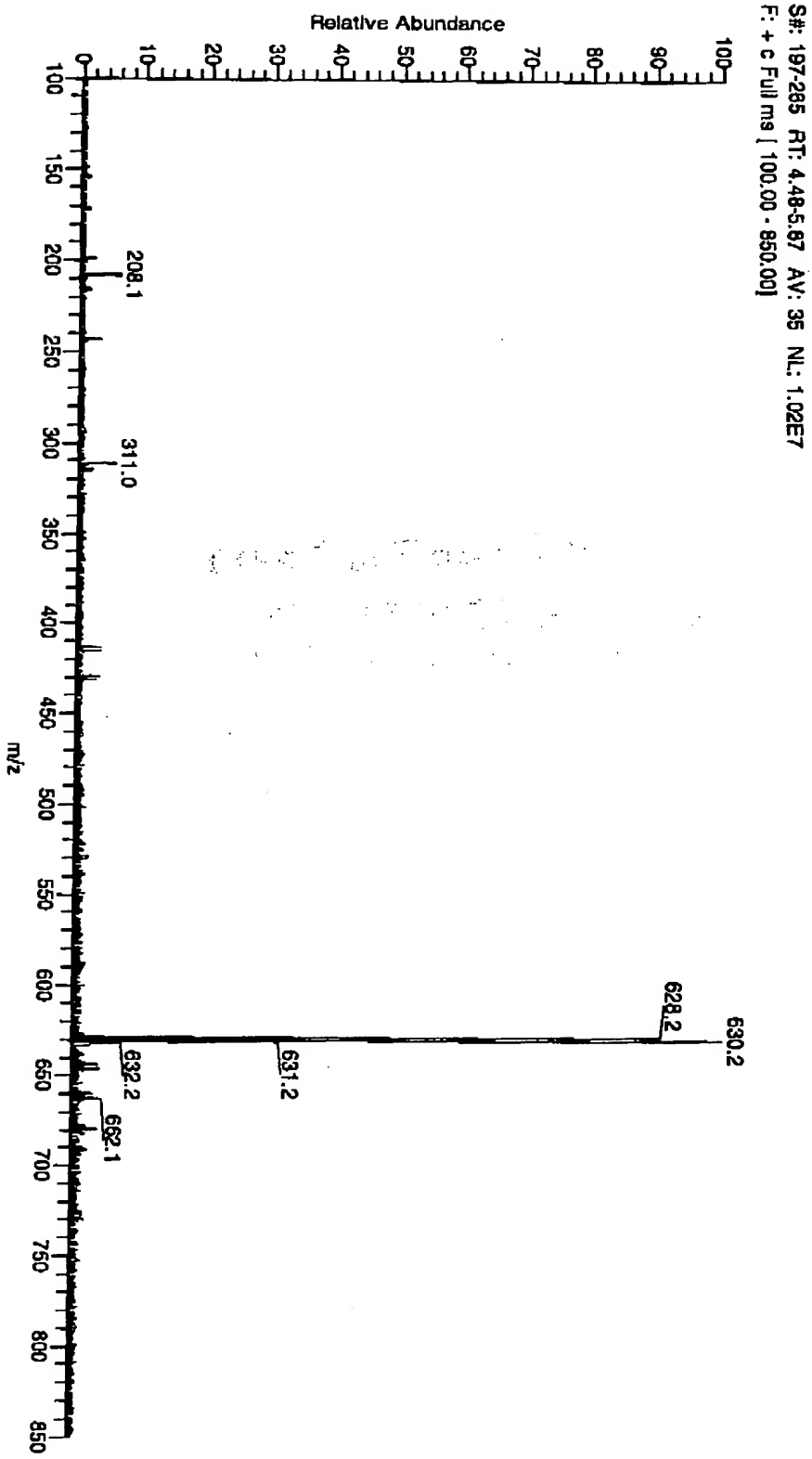


Figure 5 L981292

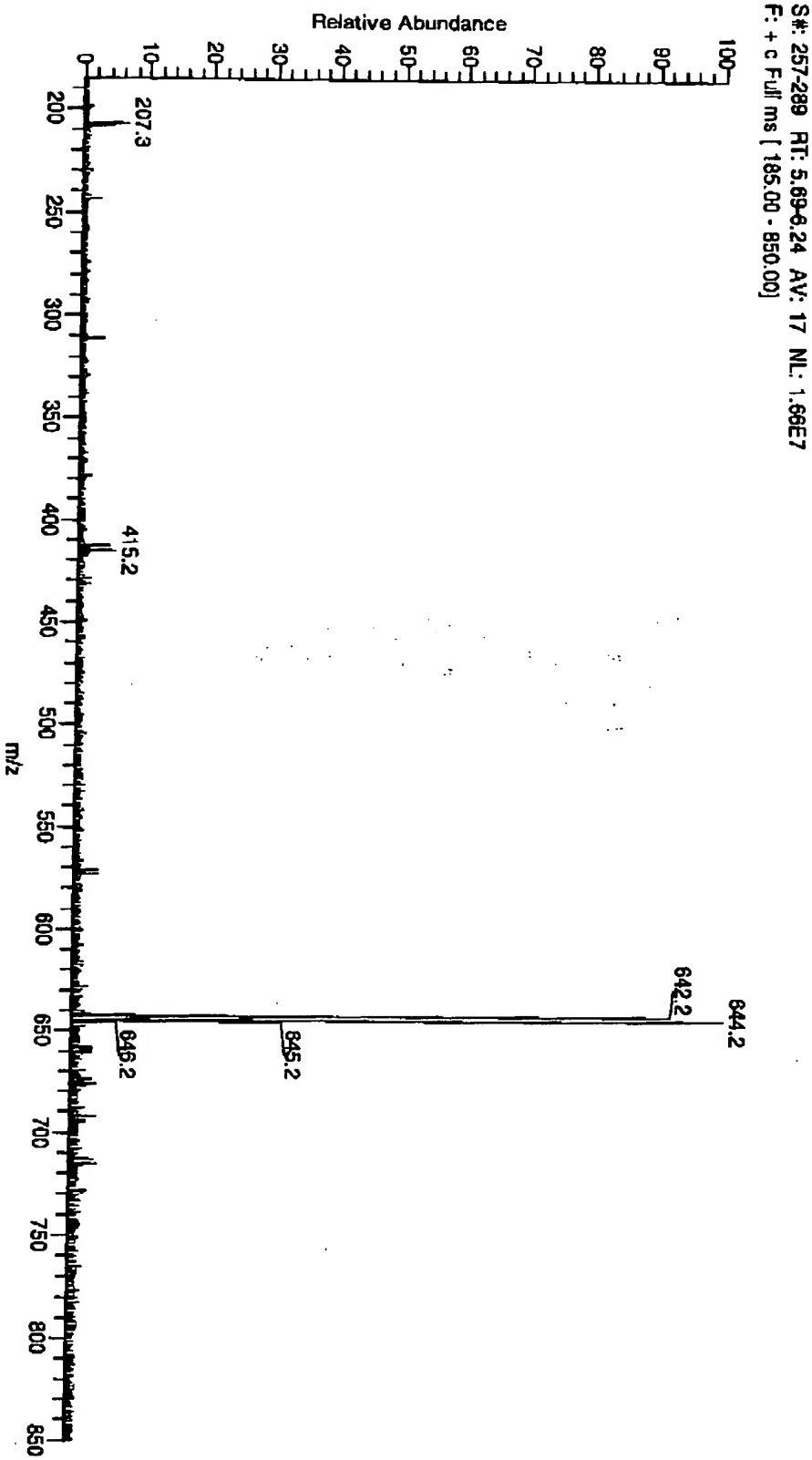


Figure 6 L981282

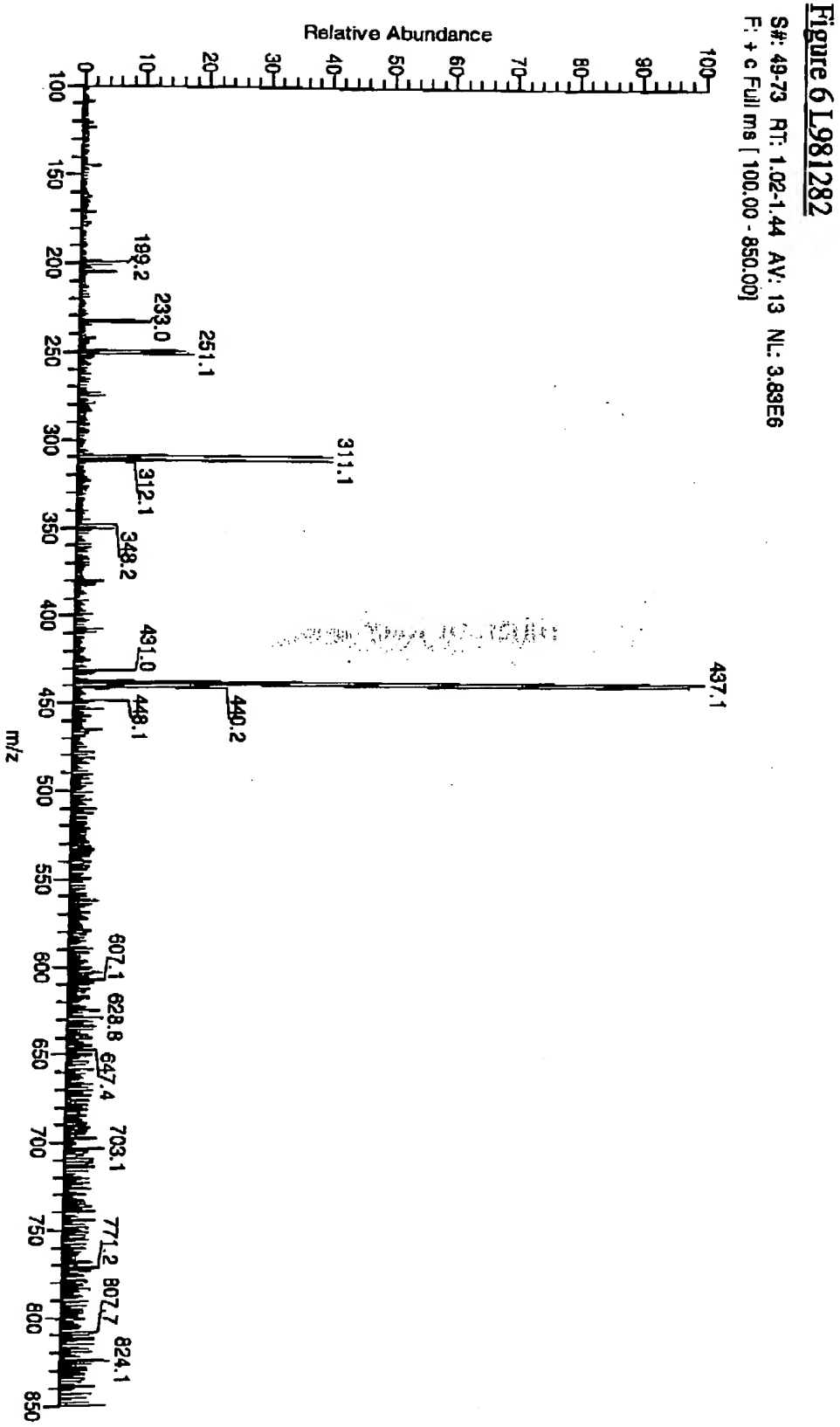


Figure 7 L981287

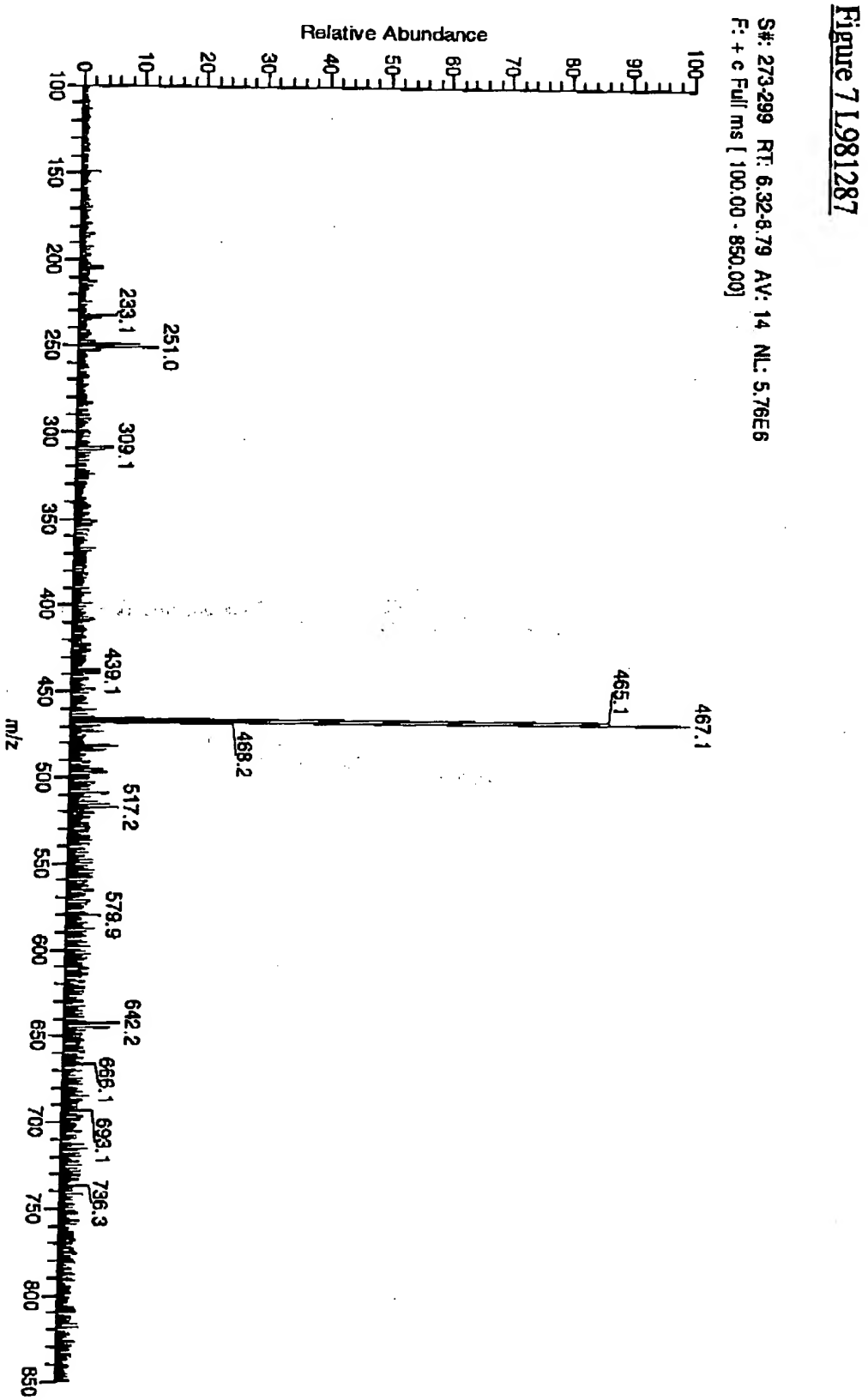


Figure 8 L981297